UNIVERSITY OF ALBERTA

DEVELOPMENT OF THE EMBRYONIC DENTITION IN *XENOPUS LAEVIS* (DAUDIN): DESCRIPTIVE AND EXPERIMENTAL STUDIES BETWEEN STAGES 54 AND 61

BY

DENIS OSWALD LAMOUREUX ©

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the requirements for the degree of DOCTOR OF PHILOSOPHY

IN

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The undersign certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled DEVELOPMENT OF THE EMBRYONIC DENTITION IN *XENOPUS LAEVIS* (DAUDIN): DESCRIPTIVE AND EXPERIMENTAL STUDIES BETWEEN STAGES 54 AND 61 submitted by DENIS OSWALD LAMOUREUX in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Oral Biology.

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Paul Scott
P.G. Scott

PS for
A. Kemp

23 December 1996

When the spirit dies there's not much left . . . a week of deliberation. Sitting here going mad . . . and Sperber walks in.

Rutherford Library
2 July 1982

To my mind it accords better with what we know of the laws impressed on matter by the Creator, that the production and extinction of the past and present inhabitants of the world should have been due to secondary causes like those determining the birth and death of the individual.

Charles Darwin
On the Origin of Species (1859)

And they were terrified and asked each other, "Who is this? Even the wind and the waves obey him?"

Mark 4:41

DEDICATION

Both to Nicole Simpson Brown whose murder sensitized our generation to the reality and wickedness of physical abuse, and to the gentle old southern cowboy who made me aware of the reality and wickedness of verbal abuse.

ABSTRACT

The transparency of *Xenopus laevis* (Daudin) tadpoles and the visibility of their developing tooth germs permit the longitudinal recording of the embryonic dentition both in normal development and following surgical manipulation.

Twenty-two tadpoles from late Stage 54 to Stage 61 (Nieuwkoop and Faber, 1967) were anesthetized daily and observed under a dissecting microscope to identify the developing tooth germs and to measure the distances between them. The development of the dentition began during late Stage 54 in the medial two-thirds of each upper jaw quadrant with the abrupt appearance of 4-8 tooth germs over 2-4 days to form the Initial Dental Row (IDR). At Stage 55 the Distal Dental Row (DDR) began slowly extending back from the IDR over the next 2 weeks. In almost every case 1-3 tooth germs developed in the interdental space between the first tooth germs in both the IDR and DDR. Each jaw quadrant contained 17-23 tooth germs (mean: 20.5) by Stage 61. Employing the criteria of tooth germ position and developmental time, 9 developmental tooth germ types and 4 developmental configurations of tooth germs were identified. Two relationships emerged in the developing dental row. First, the average distance between a pair of tooth germs varied inversely with the time it took for an interdental tooth germ to develop. Second, the average distance between the first tooth germs to appear in the dental row related directly to the number of tooth germs that later developed between them.

Five experimental procedures were performed: (1) A tooth germ was extirpated at the beginning of the development of the dentition in 35 animals. The resultant dental initiation patterns were not simply the expected dental configuration less the removed tooth germ. Rather, tooth germ number, type, position and development time were modified around the surgical site, suggesting an epigenetic or regulative element in the

developing dental row. (2) The entire middle third of the upper jaw quadrant, including the suprarostral cartilage and associated intraoral and extraoral tissues, was extirpated from 6 tadpoles at Stage 45. The surgical defect was maintained during the observation period. Dental development was normal in the remaining thirds of the jaw quadrant. (3) Sections (250-350 µm) of presumptive dental row tissue up to the overlying suprarostral cartilage were excised from one of the upper jaw quadrant thirds in 24 Stage 53 animals. This tissue was transplanted to a dorsal site between the olfactory nerves. Dental development occurred in every recoverable graft. (4) The entire presumptive dental row in 12 Stage 53 tadpoles was extirpated from the midline to the corner of the mouth. Tooth germs appeared later in the recording period and the initiation patterns did not feature the quickly appearing IDR and distally extending DDR seen in normal development. Rather, an initial dental row was quickly regenerated across the entire jaw quadrant with interdental spacing similar to the IDR. (5) Tissue recombinations were made between oral and non-oral tissues to investigate epithelial-mesenchymal interactions during early odontogenesis. Results were consistent with murine studies, as dental patterning was first established in the epithelium, suggesting the evolutionary conservation of these interactions.

The descriptive and experimental results from this study on the developing dentition in *Xenopus laevis* larvae were not consistent with the standard models for the development of the dentition--Zahnreihen, Clone or New Progress Zone theories. Instead, the Odontogenic Field Theory is proposed and suggests that dental patterning reflects the competitive establishment of dental initiation zones within an epithelial odontogenic field.

ACKNOWLEDGMENTS

It wasn't a blood covenant, but Bacardi and coke in those days were tantamount to it. And 'Big Al' Leboldus is probably gloating today, "I told you so, Froggie!" An oath never to return to school, especially dental school, was the premier stipulation of a suzerainty treaty including curses and blessings with the Universe. Yep, Bolus, our assessment back in May of 1978 was indeed judicious, and our prophetic utterances remain accurate nearly some twenty years later--"what a whole lotta of bullshit." But a funny thing happened while I was off sunning my United Nations butt on an island the zipper-heads call "the rock"--through the mystery of a gracious outpouring I came to meet I AM and the propitiatory power of the Rock's covenantal blood. In the interim, a pagan house was cleansed of vanities, idolatries and debaucheries, struggles occurred in leaving Ur and climbing up Moriah, three days were even spent behind Jonah's baleen only to be spewed up on Wreck Beach, and to complete my training--a Jobian experience with a Hosean twist including being tossed into the charismatic world *nolens volens*. It was a simple calling--show up in the library or laboratory every morning at 8 AM. And two caveats reflective of the Baconian Scrolls are the fruits of the persistence. The first is appreciating the *sufficiency* and *necessity* of theopneusticity in holding Hirschian authorial intentionality and Gadamerian textual autonomy in an antinomous relation and then suffering with the neologism 'theocosmopoetry.' The second is coming to terms with a "blood trail"--the segment polarity gene *Sonic Hedgehog*, HOX genes and their combinatorial code, and the rock record of odontogenic cusps--by tempering it with the Irenaean theodicy, resulting in a biological anthropic principle of the strongest order. And so mystery, subtlety and grace have become pillars in the post-critical epistemology that undergirds the hermeneutics through which I have the privilege of reading the Two Great Books.

But how do I describe this last part of my nineteen year adventure in the academy? Employing the literary genre and categories of the writer on the island of Patmos, it could be summarized with the metaphors of eschatological warfare: the incarnation and demonic rage of an unholy trinity--the dental staff from hell, the supervisors from hell, and the girlfriend from hell.[1] Yep, I kinda saw it all this time through . . . the ethical integrity of Tammy Faye Baaker as seen in one who could two-time on her boyfriend and pray with an unsuspecting suitor (*Dolus bonus* [yea, even in a feminist form] I am sure has never been strained so forcefully). But then the classic 'sin of the mothers' (ah, that dirty Biblical 'S' word, again, ladies) stormed in the preceding generation. And I also viewed the integrity of Jim (i.e., Baaker, not Morrison . . . yet is there really any difference other than the newly acquired fundamentalistic rhetoric?) as revealed with a purported Christian brother who offered to whisk away an engaged woman to Seattle. Well, how's that go? God does work in mysterious ways . . . yea, even through rebirth by Caesarian section, indeed! (Now that's one creation story that is going to need the editorial massaging from both apocryphal and mythical scribes . . . yet legal hermeneutics has its own demonic proficiency) But then if one has never been "house-trained" he is bound to fish off the company pier whether it be for minnows or barracudas . . . so there were no real surprises . . . so common, so crass . . . an afternoon soap in a trailer court . . . a story for Jerry Springer. And thus the Biogenetic Law is fulfilled as cockroaches . . . well they mate with . . . Yet despite the ragged edges of life and its many wickedly dark periods, lighter moments always arise. For example, who didn't have a good chortle in trying to figure out how many Golden Bear football players it takes to tackle a 148 pound 41 year old with a bad back and sore wrist? Is this the laughter of the Toronto Vineyard experience? (Well, there certainly was something "signs & wonders-ish" about it all, just ask the sister of a colleague in attendance as she acted as a messenger and prayed with a wounded heart during the public ratification of the new ontological reality) Yet one charismatic septuagenarian wasn't amused and had to be restrained from pounding on somebody's mahogany. To summarize the entire situation in one word, let us use the assessment of a wise sort-of-old indian who during the arbitration of a domestic dispute judged: "disappointment." Yes, disappointment . . . one winter great boasts of studying philosophy at Notre Dame and charges against one supposing losing his First Love . . . and a year later the chair of that university department taken to task and Revelation of a faith gone awry with confirmation by two witnesses closest and standing the longest. And so the faith of a 'Baptist Thief' reigns in his disciples from on high in the city. Yep, for some a legacy is very important . . . and for the children who have ears, let them hear.

---

[1] I am very grateful to the girlfriend from hell who inspired me to employ her epithet which was modeled off one of her former roommates--the roommate from hell.

But the genius of the Irenaean approach to theodicy is that it introduces an existential element that transcends any other manner of conceptualization, and this came into sharp focus during this PhD program. For example, one February afternoon on the steps outside the Dentistry-Pharmacy Centre I was enamoured by the piety of a plaintiff's cry, "Where are they who should be 'standing-in-the-gap' at the university?" However, I soon came to be disenchanted and experienced the vacuity of the complaint since the answer to the query was that ironically they were out playing baseball and basketball, baseball and basketball . . . bounce, bounce, bounce . . . And again, we often mock the Pauline admonition of equal yoking with the fundamentalist formalism of a Pharisee. But it is only by walking in this spiritual discontinuity that one can appreciate a modern interpolation to the Corinthian text: "What harmony is there between a yuppie and an anti-yuppie?" Finally, it is indeed 'no ordinary person' that can teach you anger, hate and betrayal . . . yet it is the Bishop of Lyon's approach as modelled by Solzhenitsyn's gratefulness to his incarceration that one can look back and say, "Thank you, oh prison, thank you." And it is another prison, this time Frankl's, within which the will for meaning is casted and refined . . . and how one cold lonely November morning I awoke hanging on simply of Jesus's words, "Feed my lambs . . . take care of my sheep . . . feed my sheep . . ." For those who have ears, let them hear.

I am grateful this chapter is over. It was long over due and would have never come to a conclusion without the support and prayers of many fine souls: My best friend Bert Robinson, my counsellor and pal Lorraine Lafranchise, my pastor Brian Glubish, my big brother Don Lewis, my editor Angela Anderson-Konrad, my encourager Keith Kowalsky, my Bloor Street confidant Chris Barrigar, my ageless dental assistant Lyn Reiter and my friends Ted and Susan Zukiwsky.

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Many thanks to Dr. Terry Davis and Dr. Bill McBlain who were instrumental in the completion of this degree and were a model of administrative excellence in upholding the integrity of the University of Alberta.

To brother Mickey--a better roomate for six years no one could ever find . . . even with the cowboy music.

And a penultimate thank you to two intellectual revolutionaries--Dr. Loren Wilkinson, professor of interdisciplinary studies at Regent College, and Dr. Warren Gallin, professor of molecular biology at this university. These two men quite unsuspectingly reconstructed my perception of the Two Great Books: the former through Polanyi, Thiselton and Kuhn; the later through the primary literature on limb development in preparation for my candidacy exam.

Finally, I want to thank Ozzie and Bernice Lamoureux, better known as Mom and Dad, who provided a happy home and a healthy respect for education. Not every child is blessed with parents whom he not only loves but also appreciates as friends. Our family meal every Sunday evening is the most excellentest of traditions. As usual they have agreed to accept responsibility for any errors or bad points contained in the present thesis, while allowing me to take credit for anything good that may come of it.

Now the fun is just about to begin . . . with or without a **real** job!

To God Be The Glory.
DOL 31 January 1997

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NOMENCLATURE AND ABBREVIATIONS

Apical: A dental term meaning toward the end of the root of a tooth.

Complete Dental Row (CDR): The entire dental row in the jaw quadrant at Stage 61. It features between 17-23 tooth germs and includes the MDR and DDR.

Dental Triplet: The arrangement of three sequential primaries in the IDR.

Distal: A dental term meaning toward the lateral aspect of the dental row (i.e., toward the back of the jaw).

Distal Dental Row (DDR): The row of tooth germs which extends out sequentially from the IDR.

Doublet Dental Configuration: The arrangement of two primaries separated by a secondary and a tertiary.

Empty Lacuna (EL): Tooth germ development stage as observed under a dissecting microscope *in vivo*. A well-circumscribed lacuna (125-150 X 100 µm), but no discernible tooth present.

Epigenetic: Biological interactions beyond the genomic level and between tissues during development (e.g., epithelial-mesenchymal tissue inductions).

Heterodonty: A term used to describe a dentition that includes different anatomical tooth types (e.g., incisors, canines, molars).

Hint of Tooth (HT): Tooth germ development stage as observed under a dissecting microscope *in vivo*. A well-circumscribed lacuna with a discernible conical tooth <25 µm.

Homodonty: A term used to describe a dentition that has only one anatomical tooth type.

Initial Dental Row (IDR): The row of tooth germs in the mesial two-thirds of the upper jaw quadrant. It appears in the mouth during the first 4 days of the development of the dentition between late Stage 54 and early Stage 55.

Initiation Zone (IZ): A critical volume of preodontogenic epithelial cells within which dental morphogenesis first proceeds.

Jaw Quadrant Length (JQL): The length of the jaw quadrant from the midline to the the corner of the mouth which is landmarked by the base of the tentacular cartilage.

Labial: A dental term meaning toward the lip.

Lingual: A dental term meaning toward the tongue.

Large Tooth (LT): Tooth germ development stage as observed under a dissecting microscope *in vivo*. A well-circumscribed lacuna with a discernible conical tooth 75+ µm.

Mesial: A dental term meaning toward the medial aspect of the dental row (i.e., toward the front or midline of the jaw).

Medium Tooth (MT): Tooth germ development stage as observed under a dissecting microscope *in vivo*. A well-circumscribed lacuna with a discernible conical tooth 50-75 µm.

Mesial Dental Row (MDR): The dental row that includes the IDR with the subsequent addition of interdental tooth germs and also the development of one or two tooth germs at its mesial end.

Null Dental Configuration: The arrangement of two primaries with no interdental tooth germ development.

Occlusal: A dental term meaning toward the biting or piercing surface of a tooth.

Odontogenic Recording Period (ORP): The period between late Stage 54 and Stage 61 in larval *X. laevis* in which the development of the dentition can be recorded *in vivo*.

Odontogenic Field (OF): A field of oral epithelial cells within which initiation zones are competitively established, resulting in the development of the complete dental row.

Palatal: A dental term meaning toward the palate.

Partial Lacuna (PL): Tooth germ development stage as observed under a dissecting microscope *in vivo*. The tooth germ lacuna is barely discernible.

Polyphodonty: A dentition found in most lower tetrapods in which the teeth are continually replaced throughout life.

Primary Tooth Germ (Primaries): The first tooth germs that appear in the dental row. In most cases interdental tooth germs later develop between them.

Secondary Tooth Germ (Secondaries): The first tooth germ that develops between two primaries.

Small Tooth (ST): Tooth germ development stage as observed under a dissecting microscope *in vivo*. A well-circumscribed lacuna with a discernible conical tooth 25-50 µm.

Solitary Dental Configuration: The arrangement of two primaries separated by a secondary.

Surgical Triplet: The arrangement of three sequential IDR primaries after which the central primary has been extirpated.

Tertiary Tooth Germ (Tertiaries): The tooth germ that develops between a primary and a secondary.

Tooth Germ Occupancy Time: The time in days a tooth germ is present at a locus during the ORP.

Tooth Germ Development Time: The time in days it takes for an interdental tooth germ to appear after the establishment of its two neighbouring tooth germs.

Triad Dental Configuration: The arrangement of two primaries separated by a single central secondary and two tertiaries.

Triplet Length: The distance between the centres of the lateral primaries of a triplet.

Triplet Tooth Germ Number: The number of tooth germs between the lateral primaries of a triplet.

CHAPTER 1
INTRODUCTION

The developing dentition offers an excellent system for examining the mechanisms controlling organogenesis. Investigations recording embryonic tooth initiation patterns in reptiles have been employed to formulate the most influential theories on the development of the dentition. However, observational studies require experimental inquiry to test the hypotheses, and to date there is little if any relevant experimental work supporting the modern development models. In this century, five major theories have attempted to explain the development of the embryonic dentition--Distichy, Dental Field, Zahnreihen, Clone and New Progress Zone.

A. THEORIES ON THE DEVELOPMENT OF THE DENTITION

(1) DISTICHY THEORY

Loomis (1900) and Harrison (1901) noted that in short sequences in the jaws of fossil reptiles and amphibians every alternate tooth position was empty. In studying the dentition of *Sphenodon*, Harrison concluded that there were two alternating dentitions. Bolk (1912, 1916, 1922) later developed this notion in his Distichy Theory, suggesting that the dental lamina had two sites of tooth formation--the endostichos at the apical margin of the dental lamina and the exostichos, an occlusally positioned site, on the labial aspect of the dental lamina **(Figure 1-1)**. Unfortunately, Bolk worked with older embryos of *Crocodylus*, and his student Woerdeman (1919, 1921) later demonstrated that all teeth begin their development at the free margin of the dental lamina. However, Bolk's tooth alternation paradigm significantly shaped theories on the development of the dentition that later followed it.

(2) DENTAL FIELD THEORY

Butler's (1939, 1956, 1978, 1995) Dental Field Theory proposed that different embryological fields account for the differences in tooth morphology. More specifically, he suggested that a series of identical dental primordia developed in accordance with the dental field (e.g., incisor, canine or molar fields) in which they were positioned (**Figure 1-2**). This program accounted for heterodonty or secondary dental pattern--i.e., the shape and size of each tooth. However, it did not explain the mechanism that established the primary dental pattern (i.e., the position and the number of these primordia in the jaws), nor did it explain the nature of the field. Butler only assumed that the primordia were equivalent and evenly distributed throughout the jaw.

(3) ZAHNREIHEN THEORY

Edmund (1960, 1962, 1969) investigated the phenomenon of back-to-front wave replacement at alternate tooth loci in polyphyodont lower vertebrates. Employing primarily fossil evidence and incorporating Woerdeman's (1919, 1921) embryological descriptions of *Crocodylus porosus*, he proposed the Zahnreihen Theory (German for "tooth rows"). This model features two important assumptions. First, tooth initiation is a response to a morphogen released from a single generator at the front of the jaw that travels to the back, inducing successive primordia at regular temporal and spatial intervals (**Figures 1-3** and **1-4**). Second, the relationship between the replacement time (i.e., the time between eruptions of teeth at one tooth locus) and the successional time (i.e., the time it takes the morphogen to travel from one tooth locus to the adjacent locus) accounted for the pattern of the tooth replacement waves (**Figure 1-5**). Expressed as a ratio of replacement time over successional time, and termed "the Z-Spacing," this relationship has proven exceedingly attractive to some scholars since it can describe the variety of wave replacement patterns (Ewer, 1963; Osborn, 1975, 1977). For example, Z-Spacings of less than 2.0 characterized the rather rare phenomenon of front-to-back waves (e.g., agamid lizards and piranha fish), Z-Spacings of 2.0 are seen in strict alternation (e.g. Crocodilians, Red-back salamander), and Z-Spacings of greater than 2.0 have back-to-front waves and are representative of the majority of polyphyodonts. However, no experimental evidence has ever confirmed Edmund's theoretical morphogen generator or travelling morphogens.

(4) CLONE THEORY

Osborn (1970, 1971, 1972, 1973, 1974a, 1974b, 1975, 1977, 1978, 1984, 1993), in response to the Zahnreihen Theory, developed the Clone Theory. He suggested that in the embryonic jaw of the common English lizard *Lacerta vivipara* (Osborn, 1971) the earliest teeth in the mouth appeared with "varying frequency" at tooth positions 3, 5, 6, 8, 10, and 13. This pattern is *contra* the orderly sequence from the midline suggested by the Zahnreihen model. Second, Osborn *deduced* that dental initiation was from the back of the jaw to the front. Again this pattern is *contra* the front-to-back initiation order predicted by Edmund's theory. In addition, Osborn also proposed that a sphere of inhibition surrounded the developing tooth, and that it not only ensured the even spacing of teeth in the developing jaw, but could also account for the dental wave replacement patterns. Osborn (1978, 1984) later refined his thesis and suggested that "a discrete group of [ectomesenchymal] cells," termed "a clone," expands in concert with the developing jaw and gives rise to the dentition. In the lower jaw of reptiles, the entire dentition is the result of one clone that is initiated at a single site (termed the "clone determinant") in the middle of the jaw quadrant and then expands anteriorly and posteriorly. Interstitial growth between the first teeth creates space for the development of intervening teeth (**Figure 1-6**). In contrast to lower tetrapods, Osborn proposed that mammals have three clones--a posteriorly growing incisive clone, a single tooth family canine clone, and a molar clone which grows both posteriorly and anteriorly. The single clone of ancient reptiles according to Osborn was reduced to the canine, and the incisor and molar clones evolved during mammalian evolution. The clone theory claimed experimental support when Lumsden (1978, 1979) transplanted murine presumptive M1 tissue (E12--dental lamina stage) intraocularly and an entire and recognizable molar series of three teeth developed. Lumsden, though, never clearly established the nature of a clone; i.e., whether it was a "two-layer structure" composed of epithelial and mesenchymal cells, or only mesenchymal cells.

(5) NEW PROGRESS ZONE THEORY

Westergaard and Ferguson (1986, 1987, 1990) offered the first detailed study of a developing dentition in a polyphyodont from the first initiated tooth to the first erupted and functional dentition. The dental initiation patterns in their study of the alligator *Alligator mississipiensis* clearly contradicts the Zahnreihen Theory. The first tooth to appear in both jaws develops at tooth position 3 and it is not the anterior-most tooth as predicted by Edmund. But more problematic for the Zahnreihen thesis, teeth are not initiated in strict front-to-back sequence. Instead, "interstitial teeth" develop between the "sites of primary tooth initiation" apparently in response to interstitial jaw growth. Incorporating the notion that dental patterning begins in the oral epithelium (Mina and Kollar, 1987), Westergaard and Ferguson proposed the New Progress Zone Model of dental development. However, they never explicitly outlined their theory, and it seems to include numerous mechanisms without any experimental evaluation. On the one hand, they speculated that once initiation in the epithelium at the first dental locus occurs this "early initiation stimulus", coupled with an "inhibition process," spreads in the epithelium, resulting in zones of competent epithelium capable of inducing the underlying mesenchyme into odontogenesis. On the other hand, this thesis also envisions a proliferative epithelial "progress zone" that grows in concert with the jaw growth, and that with either a cell lineage phenomenon and/or positional information phenomenon, epithelial cell division ceases, giving rise to new dental anlagen. In other words, this model for the development of the dentition features elements that are similar to the theories of Edmund and Osborn. It features both a postulated morphogen that spreads through the epithelium and an epithelial clone of growing cells.

It is important to note that with these major theories on the development of the dentition, the literature (e.g., Wake, 1980; Colyer, 1991) in the last twenty years often reflects a dichotomy introduced by Osborn (1978) between field and clone models. His paradigm emphasizes secondary dental pattern (i.e., tooth shape) more than primary pattern (i.e., tooth position in the jaw). According to Osborn, axiomatic differences between these models include: (1) All dental primordia in the field model are equivalent, and a field substance is required to determine the final shape of a primordium. In contrast, the clone model suggests that all primordia are different, and that final shape has largely been determined as soon as a primordium is initiated. (2) In the field model, dental shape is induced and controlled from outside. However, final shape in the clone model is self-generated and intrinsic. (3) Shape gradients in the field theory reflect the gradient of field substances. But in the clone theory, shape gradients are a function of clone growth as it "unfolds."

The current status of the major theories on the development of the dentition is as follows. Bolk's Distichy theory is only mentioned for historical interest. Butler's Dental Field thesis is often conflated with the Zahnreihen theory. This is due to the influence of the dichotomous paradigm between the clone and field theories introduced by Osborn (1978). However, Butler focussed on the second dental pattern and contributed little with regard to the establishment of teeth in the jaw (i.e., primary dental pattern). Recent advances in molecular biology and the demonstration of development fields and morphogen gradients may allow a sharpening of Butler's model. The Zahnreihen theory, once widely accepted in the 1960s (Goin and Hester, 1961; Cooper, 1963, 1965; Cooper *et al.*, 1970; Lawson, 1965, 1966), has come under criticism in the last twenty years. Descriptive studies on the development of the embryonic dentition in piscine, amphibian and reptilian models demonstrate that tooth initiation does not start from the tooth nearest the midline and then proceed sequentially from there (Osborn, 1971; Berkovitz, 1977a, 1977b, 1978; Berkovitz and Moore, 1974, 1975; Shaw, 1979, 1982, 1986; Westergaard, 1988a, 1988b; Westergaard and Ferguson, 1986, 1987, 1990). Moreover, Osborn (1971) and Westergaard and Ferguson (1986) even demonstrated how Edmund had skewed the embryological descriptions of Woerdeman (1919, 1921) to fit his model. However, the influence of the Zahnreihen theory still remains (Kieser *et al.*, 1993). Osborn's Clone theory is the only model that claims experimental support (Lumsden, 1978, 1979), and it continues to be discussed in the literature (Keene, 1991; Osborn, 1993; Butler, 1995). Yet recent work reveals that dental patterning is first established in the epithelium (Mina and Kollar, 1987; Kollar and Mina, 1991) and not in the ectomesenchyme as suggested by the Clone theory. As a result, the New Progress Zone theory is often cited in the current literature (Keene, 1991; Kieser *et al.*, 1993, Osborn, 1993; Butler, 1995). This theory, though, is built only on descriptive evidence and does not offer a definitive mechanism as it features both clone and field elements. Therefore, the current state of the theories on the development of the dentition finds the Clone and New Progress Zone models as the most influential paradigms. The Zahnreihen theory has nearly lost its earlier authority. The Dental Field thesis is rarely discussed in these studies because they usually are limited to homodonts and this model relates to heterodonty.

In sum, a review of the major theories on the development of the dentition in this century offers two important observations. First, other than for Lumsden's (1978, 1979) work, these models lack hard experimental evidence. This fact was appreciated early by Butler (1939), later affirmed by Osborn (1971), and recently acknowledged by Westergaard and Ferguson (1986, 1987, 1990). Descriptive studies, though valuable in scientific investigation, offer limited information with regard to the mechanisms operating in developing biological systems. Second, Wolpert (1971) asserted that "it should be a morphogenetic maxim to avoid inferring developmental mechanism from final form or pattern," and most of the models for the development of the dentition disregard this principle. The tooth replacement phenomenon in adult lower tetrapods significantly influenced the developmental theories of Bolk, Edmund and Osborn. Similarly, heterodonty shaped Butler's theory.

B. THESIS OVERVIEW

This thesis offers both descriptive and experimental studies on the development of the embryonic dentition in the tadpole of the South African Clawed Frog *Xenopus laevis* (Daudin). In particular, this study tests the predictions of theories on the development of the dentition still cited in the modern literature--Zahnreihen, Clone and New Progress Zone models.

Chapter 2 is descriptive. It offers the first *in vivo* study of the developing embryonic dentition ever reported. The transparency of *X. laevis* tadpoles and the visibility of their developing tooth germs in the upper jaw permits the longitudinal recording of the development of the dentition between Stages 54 and 61 (Nieuwkoop and Faber, 1967). During this 15-20 day period, measurements were made of the positions of the tooth germs in the developing dental row and of the length of the growing jaw. A classification of the tooth germs into nine development types was based on the position of the tooth germs relative to one another and their time of appearance in the dental row. Two specific issues are investigated in this chapter.

First, this longitudinal study invites a comparison to Shaw's (1979, 1982, 1986) extensive investigation of the development of the embryonic dentition in *X. laevis*. To date, all studies on the early dentition including Shaw's have been based on serial reconstruction data. As a result, it will be possible to note the differences between that method and the longitudinal approach.

Second, the early dental initiation patterns in the tadpole are compared to those predicted by the more recent theories on the development of the dentition. The Clone and the New Progress Zone models are intimately related to jaw growth. As a consequence, the developing dentition is expected to correlate with the growth of the jaw. Moreover, both these theories postulate that the teeth in the first tooth row appear in sequence, developing out both anteriorly and posteriorly from the first tooth initiated in the jaw quadrant. Teeth between those in the first row are also expected in response to interstitial growth. According to the Zahnreihen theory, the first tooth to develop is nearest the midline and dental development proceeds sequentially from that locus. No interdental development between the tooth germs of the first row is predicted. In addition, the development of the dentition is not directly related to jaw growth in Edmund's model.

Chapter 3 is also descriptive. It employs data collected in the previous chapter to investigate the dynamic involved in the development of the dental row. The longitudinal approach and the accessibility of the tadpole dentition allows the position of tooth germs to be measured in the developing dental row. No study has previously gathered such data. A number of relationships will be examined which include: (1) the length of the dental row and the number of tooth germs in the dental row, (2) the distance between first tooth germs to appear in the jaw and the number of tooth germs that later develop between them, and (3) the distance between two tooth germs and the length of time it takes for an interdental tooth germ to develop between them. This chapter then proposes a model for the development of the dentition based on the descriptive data collected in this and the previous chapter--the Odontogenic Field Theory.

Chapter 4 is experimental. The notion that inhibitory zones surround developing teeth was first discussed by Gillette (1955). These theoretical zones are a significant mechanism in the Clone and New Progress Zone models. However, there has never been an experiment designed and performed to confirm their existence. At best, Westergaard and Ferguson (1990) calculated from their descriptive study that the inhibitory zone in the embryonic alligator dentition is 300 µm. Osborn (1971) speculated that the extirpation of two adjacent tooth germs should release the inhibitory zone around these teeth and cause the premature initiation of the neighboring teeth.

The tadpole model permits for the first time the opportunity both to remove a tooth germ early in the development of the dentition and to observe the impact this procedure has on the developing dental row in the immediate region for 2-3 weeks after the surgery. Three simultaneously appearing tooth germs (termed a 'triplet') in the first dental row were identified. The middle tooth germ was extirpated and dental development was followed in the triplet. The number, developmental type and appearance time of the tooth germs in the triplet were recorded and compared to that of triplets identified in the normally developing dental row of the tadpoles recorded in Chapter 3.

The tooth germ extirpation experiment examines two issues. First, it investigates the existence of an inhibitory zone around a developing tooth. If such a zone is present, then removing a tooth should disrupt it, changing the expected spatial and temporal pattern of the adjacent interdental tooth germs. This result would point to a regulative or epigenetic element in the development of the dentition. However, if the dental development patterns in experimental triplets emerge as predicted in normal development, then three interpretations are possible: (1) the fate of the interdental tissue was determined prior to tooth germ extirpation, (2) the inhibitory zone never receded after the removal of the tooth germ, (3) or dental inhibitory zones do not exist. Second, the tooth germ extirpation results are considered in the light of the theories on the development of the dentition. The Clone and New Progress Zone models incorporate the notion of dental inhibitory zones and predict regulative dental patterns to emerge after the extirpation of a tooth germ. That is, dental initiation is locally controlled. In contrast, the Zahnreihen theory predicts the front-to-back sequential initiation of tooth germs in the triplet. More specifically, dental initiation is governed by an overarching mechanism originating from the front of the jaw.

Chapter 5 is also experimental. Three extirpation experiments were designed specifically to investigate the Clone, New Progress Zone and Zahnreihen theories. The development of the dentition was recorded *in vivo* in all these experiments after the surgery. In the first procedure, the middle third of the jaw quadrant was excised three weeks prior to the first histological evidence of odontogenesis. When larvae first opened their mouths (6-7 days post-fertilization), an anterior defect was created in the upper jaw that included the oral epithelium and mesenchyme, the overlying suprarostral cartilage, and the dorsal epithelium and mesenchyme. According to the Zahnreihen theory, the surgical site should interrupt the passage of the initiatory morphogens from their origin in the anterior third of the jaw quadrant to the posterior third. That is, no teeth are expected to be initiated in the distal third of the jaw in these experimental animals. For the Clone theory, the surgical defect should interfere with the growing clone of ectomesenchymal cells. The first teeth in the tadpole jaw quadrant appear in the medial two thirds, suggesting that the clone determinant (the site where the clone is first organized and where the first tooth germ appears) is in this region. As a result, dental development is not expected in the distal third of the jaw. For that matter, if the clone determinant is in the middle third of the jaw, then no tooth development is expected in either the remaining medial or distal thirds. And if the determinant is in the distal third of the quadrant, then the medial third should not develop teeth. The New Progress Zone theory bears similarities to the two previous models in that from the first dental locus (which is also near the front of the jaw) an "early initiation stimulus" spreads in the epithelium and that a proliferative epithelial "progress zone" expands in concert with jaw growth. Regardless of which mechanism is most determinative, the extirpation of the middle third of the jaw quadrant should interrupt the passage of any signal or invasion of dental epithelial cells to the distal third, and no teeth would be expected in this segment of the jaw. And if the first dental locus is in the middle third of the jaw, then neither the medial or distal jaw segments will have teeth. Finally, both the Clone and New Progress Zone theories are intimately related to jaw growth. However, the possibility exists that a clone of ectomesenchymal cells or a progress zone of epithelial cells develops in a "channel" independent of jaw growth. Despite this possibility, the expected results for middle third jaw quadrant extirpations remain identical.

In the second extirpation experiment, presumptive dental tissue was excised from the medial, middle and distal thirds of the jaw quadrant and grafted to a dorsal site between the olfactory nerves. The graft tissue included the oral epithelium and its associated mesenchyme to the overlying suprarostral cartilage. The surgical procedure was performed at late Stage 53, about 4-5 days before the histological evidence of odontogenesis. Dental development in the grafts and the surgical quadrant was recorded daily. According to the Zahnreihen theory, grafts from the middle and distal thirds would not have received the initiatory morphogen at the time of surgery, and as a consequence they should not develop teeth. Similarly, no dental development in the distal third grafts is predicted by the Clone and New Progress Zone models since the graft tissue was taken prior to the growing/invading ectomesenchymal clone/epithelial progress zone. However, the site of the clone determinant/first dental locus will determine whether teeth are found in either the medial or middle third grafts.

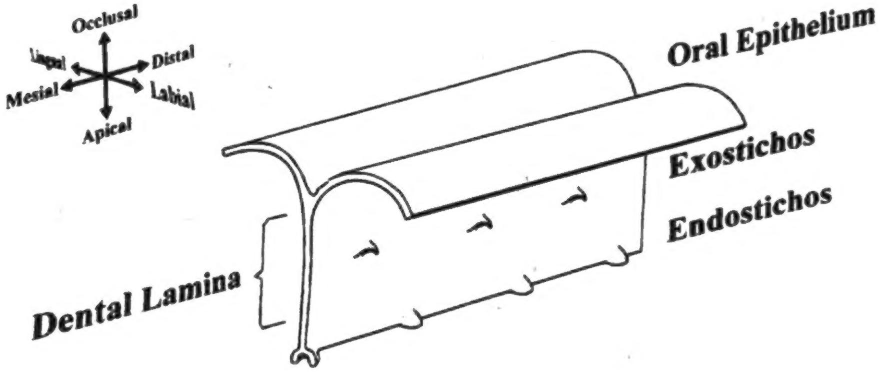
In the final extirpation experiment, the presumptive dental tissue was excised from the entire jaw quadrant. The oral epithelium and its associated mesenchyme to the overlying suprarostral cartilage were removed at late Stage 53, about 4-5 days prior to the first histological evidence of odontogenesis. The dental patterns of the regenerated oral tissue were recorded daily. In the Zahnreihen theory, it is assumed that since dental initiation is dependent upon a morphogen, it is necessary first to regenerate the morphogen generator claimed to be near the midline before dental initiation can proceed. As a result, dental patterning in these experimental jaws should follow that expected by Edmund's theory, a front-to-back initiation sequence. Similarly, both the Clone and New

Progress Zone models begin the development of the dentition from one site, and it is tempting to suggest that the renewed patterns should also mirror normal development. However, both these theories are intimately related with the growing jaw and the regeneration of the dentition would reflect jaw growth at a later time in development. Nevertheless, the dental initiation patterns of the regenerated tissue should provide clues to understanding the normative mechanisms in the development of the dentition.

Chapter 6 moves away from questions concerning the development of the dentition to focus on the initiation of the individual tooth. Most of the studies in this area have employed the mouse model, and they have established the existence of a complex sequence of reciprocal epithelial-mesenchymal interactions in dental development (Kollar and Baird, 1969, 1970a, 1970b; Thesleff, 1977; Kollar and Fisher, 1980; Lemus *et al.*, 1986a, 1986b, Kollar and Mina, 1991; Thesleff *et al.*, 1995a, 1995b). In particular, Kollar and Mina (1987) concluded that the early oral epithelium initiates dental development. A signal from the epithelium first instructs the ectomesenchyme to form a dental papilla. This induced tissue then instructively signals the oral epithelium. This chapter investigates the nature of these interactions during early odontogenesis in the tadpole. Various recombinations at different times in early development were made between the epithelium and the mesenchyme from oral and non-oral tissues. These recombination experiments attempted to determine whether dental patterning is also first established in the oral epithelium. Such a finding would be consistent with the New Progress Zone model, but in opposition to the Clone model that suggests that dental patterning resides in an ectomesenchymal clone of cells. These results are compared to murine studies, and the evolutionary conservation of these epithelial-mesenchymal interactions is considered.

The final chapter of this thesis evaluates the modern theories on the development of the dentition in the light of the descriptive and experimental evidence gathered on the developing embryonic dentition in larval *X. laevis*. This evidence is then employed to argue for a new developmental model--the Odontogenic Field Theory.

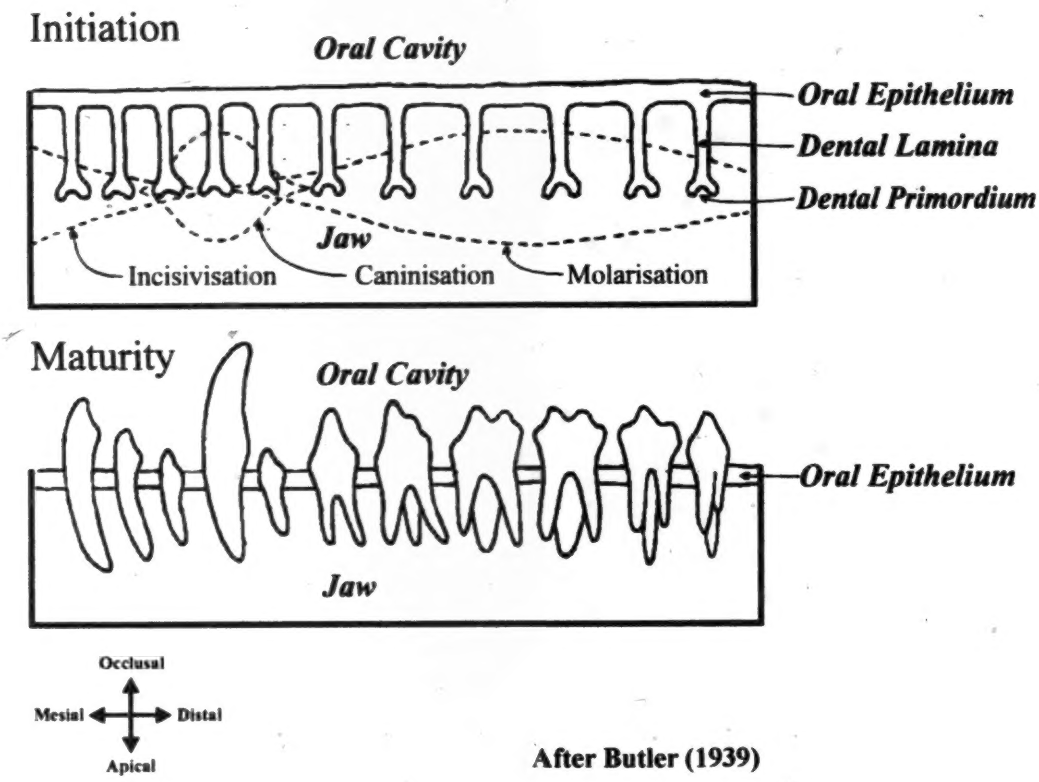
Figure 1-1.
Distichy Theory


Occlusal
Lingual
Distal
Mesial
Labial
Apical
Oral Epithelium
Exostichos
Endostichos
Dental Lamina

After Edmund (1960)

Figure 1-1. The Distichy Theory suggests that the dental lamina has two sites of tooth formation--the endostichos at the free margin of the dental lamina and the exostichos, an occlusally positioned site, on the labial aspect of the dental lamina. As teeth develop they migrate occlusally toward the oral cavity where they erupt and later exfoliate.

Figure 1-2.
Dental Field Theory


Initiation
Oral Cavity
Oral Epithelium
Dental Lamina
Dental Primordium
Jaw
Incisivisation
Caninisation
Molarisation
Maturity
Oral Cavity
Oral Epithelium
Jaw
Occlusal
Mesial
Distal
Apical

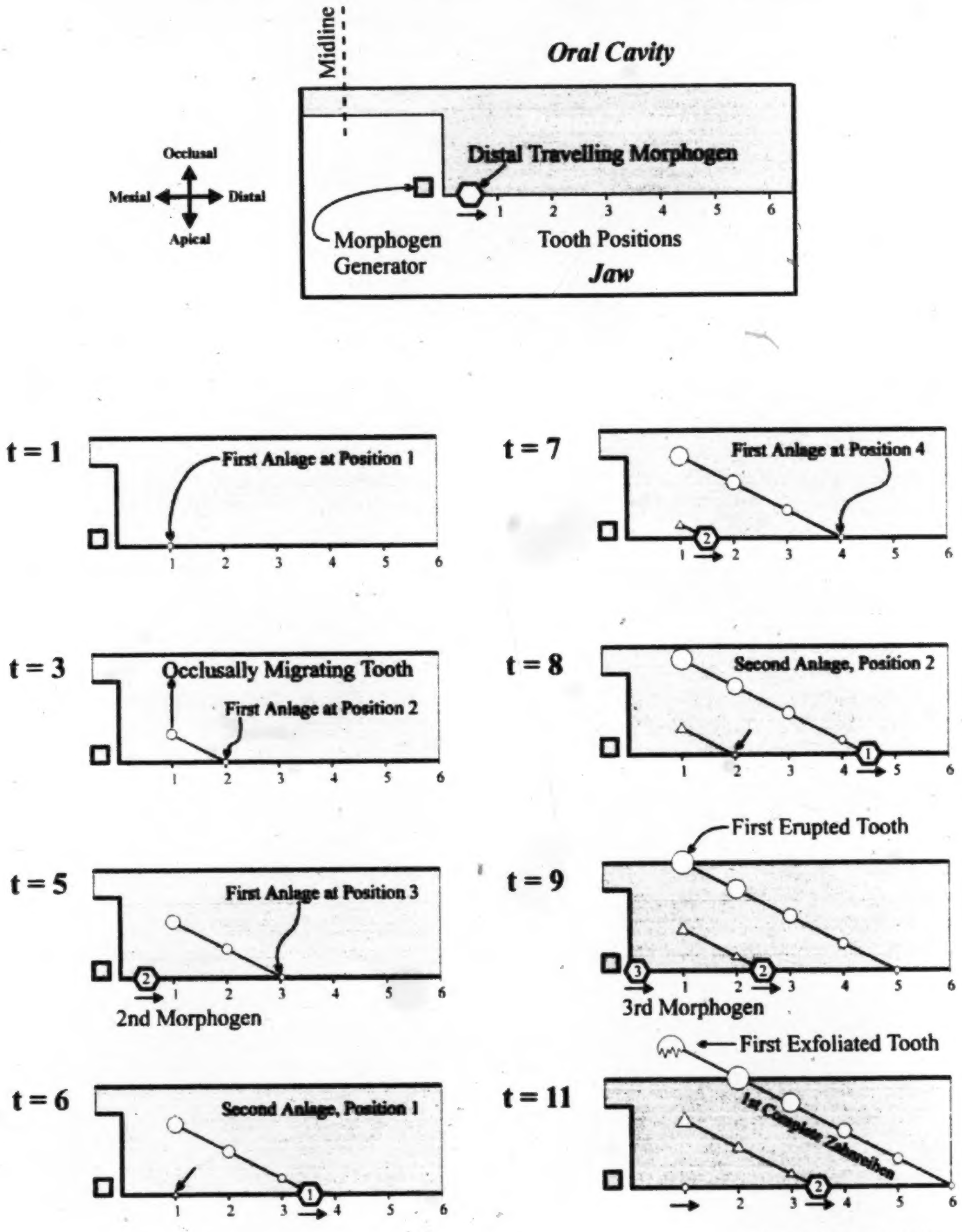
After Butler (1939)

Figure 1-2. The Dental Field Theory suggests that a series of identical dental primordia are initiated in jaw and that these develop in accordance with the dental field (e.g., incisor, canine or molar fields) in which they are positioned.

Figure 1-3. The Zahnreihen Theory suggests that a morphogen generator positioned near the midline of the jaw releases an initiating signal that travels posteriorly along the free margin of the dental lamina and initiates successive teeth at regular temporal and spatial intervals. All the teeth initiated by the same morphogen constitute a single Zahnreihen or "tooth row." As teeth develop they migrate occlusally toward the oral cavity where they erupt and later exfoliate.

Figure 1-3.

Zahnreihen Theory: Dental Initiation

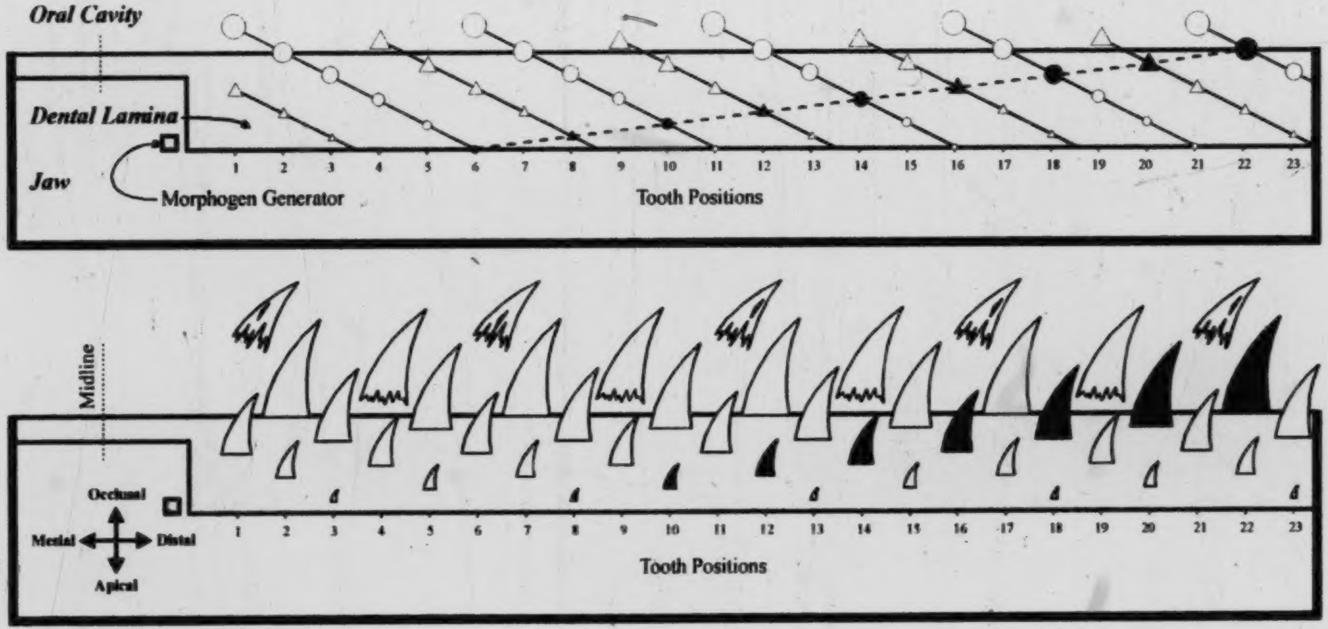

Midline
Oral Cavity
Occlusal
Mesial
Distal
Apical
Distal Travelling Morphogen
Morphogen Generator
Tooth Positions
Jaw
1
2
3
4
5
6
t = 1
First Anlage at Position 1
t = 3
Occlusally Migrating Tooth
First Anlage at Position 2
t = 5
First Anlage at Position 3
2nd Morphogen
t = 6
Second Anlage, Position 1
t = 7
First Anlage at Position 4
t = 8
Second Anlage, Position 2
t = 9
First Erupted Tooth
3rd Morphogen
t = 11
First Exfoliated Tooth
1st Complete Zahnreihen

After Edmund (1960)

Figure 1-4. The Zahnreihen Theory suggests that its dental embryological model explains the common phenomenon in polyphyodonts of back-to-front wave replacement of teeth at alternate tooth positions. The darkened teeth at alternate tooth positions 6-22 show a back-to-front replacement wave. Over time this series of teeth will give the epiphenomenon of a "wave" travelling from the back of the jaw to the front.

Figure 1-4.

Zahnreihen Theory:
Back-to-Front Dental Wave Replacement at Alternate Tooth Loci


Oral Cavity
Dental Lamina
Jaw
Morphogen Generator
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Tooth Positions
Midline
Occlusal
Medial
Distal
Apical
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Tooth Positions

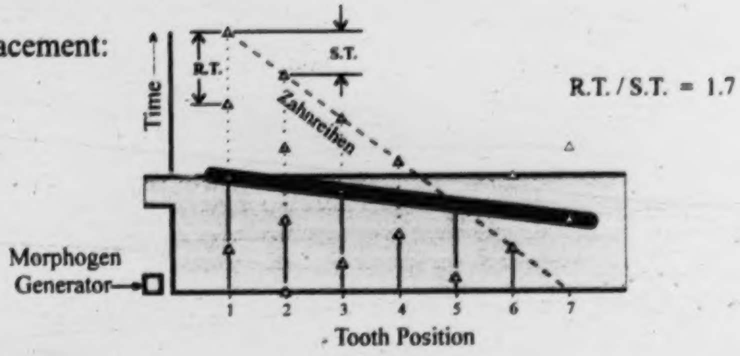
After Edmund (1960)

Figure 1-5. The Zahnreihen Theory suggests that the relationship between the replacement time (i.e., the time between eruptions of teeth at one tooth locus [R.T.]) and the successional time (i.e., the time it takes the morphogen to travel from one tooth locus to the adjacent locus [S.T.]) and accounts for the pattern of the tooth replacement waves in polyphyodonts. Z-Spacing (Z) is the ratio of replacement time over successional time (R.T./S.T). Z-Spacings less than 2.0 characterize the rather rare phenomenon of front-to-back waves (e.g., agamid lizards and piranha fish). Z-Spacings which are 2.0 are seen in strict alternation (e.g. crocodilians, red-back salamander). Z-Spacings greater than 2.0 have back-to-front waves and are representative of the majority of polyphyodonts.

Figure 1-5.
Zahnreihen Theory: Dental Wave Replacement Patterns

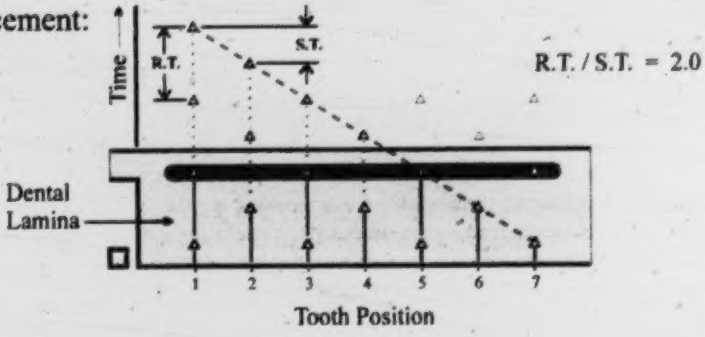
Front-to-Back Replacement:

Z - Spacing < 2.0


Time
R.T.
S.T.
Zahnreihen
R.T. / S.T. = 1.7
Morphogen Generator
1
2
3
4
5
6
7
Tooth Position

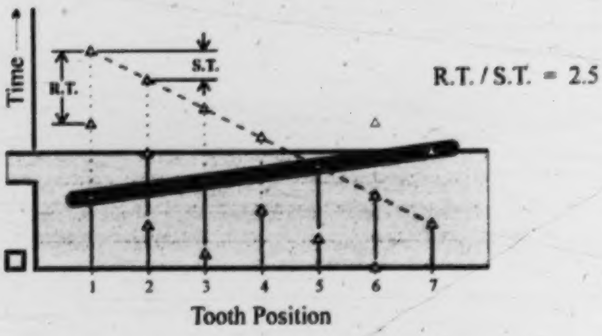
Strict Alternate Replacement:

Z - Spacing = 2.0


Time
R.T.
S.T.
R.T. / S.T. = 2.0
Dental Lamina
1
2
3
4
5
6
7
Tooth Position

Back-to-Front Replacement:

Z - Spacing > 2.0


Time
R.T.
S.T.
R.T. / S.T. = 2.5
1
2
3
4
5
6
7
Tooth Position

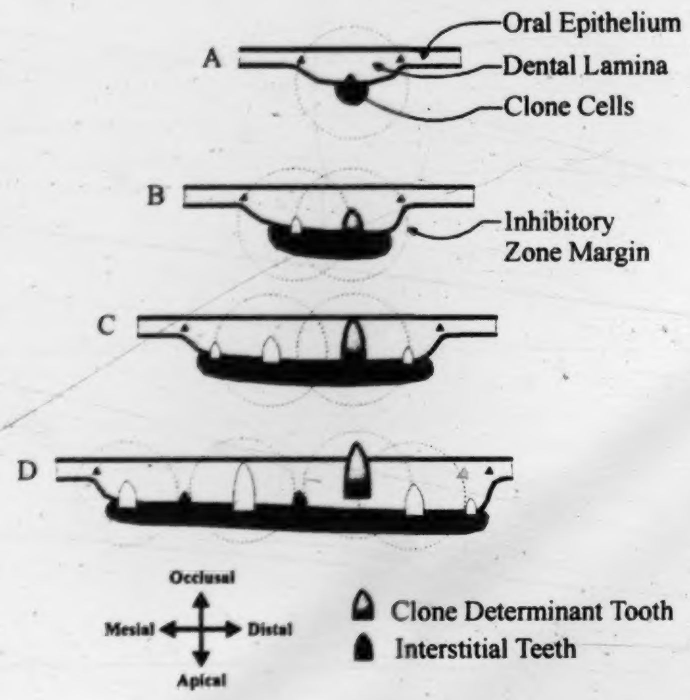
Occlusal
Mesial
Distal
Apical
Tooth
Lost Tooth

R.T. = Replacement Time

S.T. = Successional Time

After Osborn (1975)

Figure 1-6.
The Clone Theory


Oral Epithelium
A
Dental Lamina
Clone Cells
B
Inhibitory
Zone Margin
C
D
Occlusal
Mesial
Distal
Apical
Clone Determinant Tooth
Interstitial Teeth

After Osborn (1977)

Figure 1-6. The Clone Theory suggests that the dentition arises from a discrete group of ectomesenchymal cells (termed a "clone") which expand in concert with the developing jaw. In the lower jaw of reptiles, the entire dentition is the result of one clone that is initiated at a single site (termed a "clone determinant") in the middle of the jaw quadrant (**A**) and then expands anteriorly and posteriorly (**B-D**). Interstitial growth between the first teeth creates space for the development of intervening teeth. A sphere of inhibition surrounds the developing tooth and ensures the even spacing of teeth in the developing jaw.

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CHAPTER 2

TOOTH GERM INITIATION PATTERNS IN THE EMBRYONIC DENTITION: A LONGITUDINAL STUDY IN *XENOPUS LAEVIS* (DAUDIN) BETWEEN STAGES 54 AND 61

A. INTRODUCTION

Studies investigating embryonic dental initiation patterns in reptiles have been used to construct the most influential theories on the development of the dentition. Unfortunately, there are only a limited number of these investigations and none of them are longitudinal studies.

Edmund (1960, 1962, 1969) integrated his findings on tooth replacement in both extant and fossil reptilian jaws with his interpretation of Woerdeman's (1919, 1921) embryological study of *Crocodylus porosus*. He concluded that teeth in reptiles are initiated in the front of the jaw and that tooth initiation progresses sequentially in a posterior direction.

Osborn (1971) observed that the first teeth to develop in the lower jaw of the common English lizard *Lacerta vivipara* are rudimentary teeth, appearing with varying frequency at final tooth positions 3, 5, 6, 8, 10 and 13. He deduced that early in the development of the dentition a first row of teeth is initiated from the back of the jaw to the front. Interstitial growth then separates the teeth in this "initial row," creating space for the development of an "intervening row." New teeth are also added sequentially and distal to the first row.

Berkovitz (1977a, 1977b, 1978) noted that embryonic tooth initiation patterns are different between the upper and lower jaws of the rainbow trout *Salmo gairdneri*. In the mandible, the first tooth arises at tooth position 3. From this site new teeth develop both anteriorly and posteriorly in a nearly perfect alternating sequence with the odd-numbered teeth arising before the even-numbered teeth. In the upper jaw, the first tooth on the premaxilla appears at position 4, and from this site the development of the dentition proceeds sequentially in both directions. Finally, maxillary teeth develop in an almost perfect alternating sequence from the front of this bone to the back with the odd-numbered teeth appearing before the even-numbered teeth.

Shaw (1979, 1982, 1986) concluded that tooth formation in larval *Xenopus laevis* begins at the back of the jaw and progresses sequentially toward the midline. This first row of teeth is made up of the even-numbered loci and it is followed by a second row at the odd-number loci. That is, the dentition in this tadpole develops *ab initio* as an alternating series.

Westergaard (1988a) observed in the slow worm *Anguis fragilis* and the sand lizard *Lacerta agilis* that tooth initiation begins near the front of the jaw (tooth positions 2 and 4, respectively), and then proceeds both anteriorly and posteriorly. Interstitial jaw growth between these "sites of primary tooth initiation" then creates space for one or two teeth. At a few sites no interdental tooth developed between the initial teeth. In the tuatara *Sphenodon punctatus*, Westergaard (1988b) noted that the even-numbered teeth develop in sequence both anteriorly and posteriorly from tooth position 4 followed by the addition of a single tooth at each interdental site except between tooth positions 1 and 2.

In the most detailed study on the development of the early dentition, Westergaard and Ferguson (1986, 1987, 1990) recorded that teeth in the alligator *Alligator* *mississipiensis* develop anteriorly in sequence from tooth position 3 in both the upper and lower jaws. Posteriorly, the dentition develops from this position with interstitial growth in both jaws. As many as five interstitial teeth may develop between a pair of initial teeth in the first row.

All previous studies on early dental pattern formation have been based on serial reconstructions of animals at different developmental stages. However, with this method it is not possible to ascertain that the teeth observed and numbered in one animal are equivalent to those recorded in another animal. The present study investigates the development of the early dentition in larval *Xenopus laevis* (Daudin) and offers for the first time the longitudinal record of the embryonic tooth initiation patterns in individual animals.

B. MATERIAL AND METHODS

Xenopus laevis tadpoles were obtained by induced breeding with human chorionic gonadotropin (HCG, Sigma Chemical Co., St. Louis, MO). At 4 PM adult females were injected with 800 IU of HCG and placed overnight with adult males which had received 400 IU of HCG at the same time. Embryos developed in gently aerated tap water dechlorinated with 1% sodium thiosulphate (2mL per liter) and maintained at room temperature. Once they were free-swimming tadpoles, at 6-8 days post-fertilization, the animals were transferred to 40 L stock tanks (approximately 200 animals/tank) with a one way constant flow (20 L/hr) of filtered (by activated carbon) and dechlorinated (treated with sodium thiosulphate) water held at a constant pH (7.8) and constant temperature (20 ± 0.5 C°). The animals were fed Tadpole Powder (Nasco, Fort Atkinson, WI) sprinkled daily over the water surface at 7 AM and 6 PM. Two varieties of snails aided in maintaining the cleanliness of the tanks. Excess detritus was removed biweekly. A 12 h photo period was maintained (7 AM to 7 PM).

Twenty-eight precocious tadpoles at Stage 53 (Nieuwkoop and Faber, 1967) were separated from the stock population and placed into groups of four in 10 L brown buckets under the aforementioned conditions. Incisions (1 mm) on the dorsal skin between the olfactory nerves served to identify the tadpoles. The animals were anesthetized daily with 2% aminobenzoic acid ethyl ester (MS 222, Sigma Chemical Co., St. Louis, MO), and their mouths were opened with the aid of a retractor fabricated from orthodontic ligature wire. The positions of the developing tooth germs were recorded using a graticule mounted on a binocular dissecting microscope.

The transparency of *X. laevis* tadpoles and the horizontal orientation of their developing tooth germs permits the longitudinal recording of the first 15-20 days of the development of the dentition, the Odontogenic Recording Period (ORP). Recording began during mid-Stage 54 about 2-3 days before the first evidence of odontogenesis under the dissecting microscope. It continued until Stage 61 when dramatic metamorphic changes in head morphology and increased tissue opacity made accurate recording impossible. **Table 2-1** defines the stages of tooth development which could be observed *in vivo* under the dissecting microscope. These stages were confirmed histologically in stock animals between Stages 54 and 61. The tissue was fixed in formalin, demineralized with a formic acid and sodium citrate solution, sectioned at 10 μm and stained with haematoxylin and eosin.

Animals were sacrificed by anesthetic overdose (MS 222) at the end of the ORP. A strip of oral tissue containing the left and right dental rows was dissected from the underlying cartilage and examined under a tissue culture microscope to determine the final tooth count. The tooth position in the dental row was established counting from the tooth germ nearest the midline. [Note: The dental terms 'mesial' and 'distal' are employed in this paper to refer to the 'medial' and 'lateral' aspects of the dental row in each jaw quadrant, respectively (Bitgood and McMahon, 1995; Mina, *et al.*, 1995). 'Labial' means 'toward the lip' while 'palatal' indicates 'toward the palate.'].

C. RESULTS

(1) GENERAL FEATURES

The larval chondocranium is deemed "mature" at Stage 53, the final larval stage prior to the beginning of cranial ossification, and its anterior region is the skeletal support of the mouth (Weisz, 1945a, 1945b; Trueb and Hanken, 1992). Teeth develop only in the upper jaw in the narrow strip of mesenchyme between the oral epithelium and suprarostral cartilage (**Plate 2-1**). A dental row develops in each half of the upper jaw near the anterior margin of the cartilage, and these are separated by a distinct midline gap.

Tooth germs are initiated during late Stage 54 with the rapid appearance of the Initial Dental Row (IDR) in each upper jaw quadrant. The IDR is defined as the row of tooth germs which appears in the mouth during the first 4 days of the development of the dentition (average: 3.3 days; range: 2-4 days). It contains between 4 and 8 tooth germs and has a mean length of 1137 μm (range: 800-1500 μm). The interdental spaces between these first tooth germs accommodate interdental tooth germs which will develop later.

With the subsequent addition of these tooth germs and also the development of one or two tooth germs at the mesial end of the IDR, the IDR is then termed the Mesial Dental Row (MDR). At Stage 55, the Distal Dental Row (DDR) begins to extend out from the IDR with the sequential addition of tooth germs over the next two weeks (**Plates 2-2A** and **2-2B**). Interdental development later occurs between these first tooth germs in the DDR. By Stage 61, rapid and dramatic metamorphic change in the head begins and the Complete Dental Row (CDR) features between 17-23 tooth germs (average: 20.5. **Plates 2-3A** and **2-3B**).

(2) DEVELOPMENTAL TOOTH GERM TYPES AND DEVELOPMENTAL TOOTH GERM CONFIGURATIONS

During the ORP, nine developmental tooth germ types and four arrangements or configurations of tooth germs were recognized. These classifications were based upon the time the tooth germs appeared in the mouth and their positions relative to each other.

Primary tooth germs are the first tooth germs that appear in the dental row. They are equivalent to the "sites of primary tooth initiation" described by Westergaard and Ferguson (1990). In most cases interdental tooth germs later develop between them. Primaries are the most common and comprise about half of the tooth germs in each upper jaw quadrant. During development, a primary tooth germ may develop two teeth, resulting in the twinned primary tooth germ which is manifested once in every 9 jaw quadrants.

A secondary tooth germ is the first tooth germ that develops between two primaries, and it appears as early as Stage 56. About one quarter of all tooth germs are solitary secondaries. That is, there are no other tooth germs that develop between them and the bordering primaries. Their buccal margin is at the level of the middle third of the adjacent primaries. A solitary secondary bordered by two primaries is defined as the solitary configuration (**Plate 2-4A**). This is the most common arrangement of tooth germs in the dental row and it appears on the average 5-6 times in each jaw quadrant.

A tertiary tooth germ develops between a primary and a secondary. It appears by Stage 60 and is positioned near the palatal margin of the dental row. About one fifth of all secondaries are associated with tertiaries. Nearly all tertiaries are triad tertiary tooth germs. These arise in pairs separated by a triad secondary tooth germ and bordered by primaries. This triad configuration appears about once in each jaw quadrant (**Plate 2-4B**). The remaining tertiaries are unpaired. These develop between a doublet secondary tooth germ and a primary tooth germ, and are termed the doublet tertiary tooth germ. This results in the infrequent doublet configuration.

The failure of a new tooth germ to develop between two primaries creates the null configuration which appears within the dental row only once in every four jaw quadrants. A null also develops at the distal end of every dental row. A mesial end tooth germ appears in most dental rows and is similar to a secondary with regard to its time of appearance and position in the row. Replacement tooth germs develop late in the ORP and are directly palatal to a primary (**Plate 2-5**). About one out of every 7 primaries has a replacement at this stage of development. Finally, in contrast to Osborn (1971) and Westergaard and Ferguson (1986, 1987, 1990), no "rudimentary teeth" or abortive tooth germs were noted in this study. **Table 2-2** summarizes the incidence of the developmental tooth germ types and developmental tooth germ configurations. **Figure 2-1** schematizes dental row development in this tadpole between late Stage 54 and Stage 61.

(3) TOOTH GERM INITIATION PATTERNS

Figure 2-2 is a composite based on 44 jaw quadrants that gives the average number of days a tooth germ is present at any one tooth position regardless of its developmental type. It confirms Shaw's (1979, 1982) findings that even-numbered teeth develop before the adjacent odd-numbered teeth in this tadpole. He noted that by Stage 66 this even type pattern (ET) exists in 96% of the jaw quadrants. In the present study, 4 of the 44 jaw quadrants developed the odd type pattern (OT) by Stage 61. This composite of tooth germ occupancy over time reveals a "plateau/slope" pattern of the even-numbered tooth germs. That is, an IDR of 5-6 even-numbered tooth germs quickly appears followed by a DDR that slowly extends distally with the sequential addition of more even-numbered tooth germs during the rest of the ORP. Similarly, this "plateau/slope" pattern emerges if the occupancy time of only the primary tooth germs is considered **(Figure 2-3)**.

An examination of the dental development patterns in the jaw quadrants of individual larvae demonstrates the limits of the composite tooth germ initiation pattern (**Figure 2-2**). Only 3 of the 44 jaw quadrants bear a close resemblance to the general pattern **(Table 2-3)**. Completely overlooked are the null, doublet and triad dental configurations found in varying combinations in the other 41 jaw quadrants. As a consequence, the presence of tertiaries in the latter two configurations are not apparent. The general pattern also fails to distinguish unique and striking development patterns. For example, a pleasing symmetrical pattern is present with the four sequentially positioned dental triad configurations in one jaw quadrant (**Figure 2-4A**). Also overlooked by the composite pattern are 3 clear cases where the IDR slopes toward the midline (**Figure 2-4B**). Moreover, the general pattern suggests that IDR spans 10 tooth positions, but it may span as many as 19 tooth positions with a DDR of only 3 teeth (**Figure 2-4C**). Finally, the composite pattern fails to reveal the variability between the right and left sides of the same animal (**Figures 2-4A** and **2-4D**).

This variability in dental pattern is particularly seen in the development of the tertiary tooth germs. In 7 jaw quadrants there are no tertiaries and only secondaries are found between the primaries. In contrast, 13 animals have 4 or more tertiaries. As many as 8 tertiaries are present in one jaw quadrant. Further, the position of the triad and doublet configurations in the dental row also reflect this dental pattern variability. These configurations appear in different combinations anywhere between tooth positions 3 and 16.

The initiation patterns of the first tooth germs in the mouth are also variable. The number of tooth germs initiated on the first day of the ORP varies from 1 to 4 tooth germs (1 [19/44], 2 [16/44], 3 [6/44], 4 [3/44]). Dental rows with only one tooth germ initiated on the first recording day can have it appear at eight different tooth positions--4, 6, 7, 8, 9, 10, 11 and 12. Moreover, there is no apparent direction to the order of initiation of the tooth germs in the IDR (**Figure 2-5**). Animal 5 has back-to-front initiation from one distal site (tooth position 12) while it is in both directions from a mesial site (tooth position 4) in Animal 6. Initiation in mesial and distal directions from two loci (tooth positions 4 and 10) is seen in Animal 7. A region that later accommodates eight tooth positions (5 to 13) has the coincident initiation of four tooth germs (Animal 8). No clear directionality is apparent in the initiation of IDR tooth germs in Animal 9. Finally, variability is also noted between the time the first tooth germ(s) initiated in the dental row and the external staging criteria. In 4 animals odontogenesis begins 1 day prior to Stage 55 while in 3 animals it is 4 days before this stage.

(4) JAW DEVELOPMENT

At the beginning of the ORP, the average length of the jaw quadrant is 2145 µm (range: 2000-2250 µm; SD: 65) and it increases 13.4% by Stage 61 (average final length: 2473 µm; range: 2350-2650; SD: 87). However, the average final length of the CDR is 2044 µm (range: 1775-2400 µm; SD: 130) and it far exceeds the average jaw growth of each quadrant during this period (mean: 328 µm; range: 75-575 µm; SD: 101). Similarly, the rapid establishment over 2-4 days of the IDR (average length: 1137 µm; range: 800-1500 µm; SD: 182) without a corresponding increase in jaw length (average: 71 µm; range: 0-200 µm; SD: 60) also indicates that the development of the dentition is not a *direct* response to jaw growth. Moreover, there is no relationship between the number of tooth germs that develop and either the amount of jaw quadrant growth or its final length (r: 0.04 and 0.07, respectively). However, there is a slight relationship between the final dental row length and jaw quadrant growth (r: 0.32). A firmer correlation (r: 0.52) exists between the final length of the dental row and the final length of the jaw quadrant.

D. DISCUSSION

This study is the first longitudinal record of the development of the early dentition.

The embryonic dental patterns in the individual *X. laevis* larvae reveal significant variability between animals. However, this variability is expressed within certain structural limits.

These results elucidate previous reports on the development of the early dentition in this tadpole. In *The Normal Table*, Nieuwkoop and Faber (1967) noted the development of three dental rows appearing at Stages 55, 59 and 60. Deuchar (1975) also acknowledged the successive initiation of three rows of tooth germs. It appears that these reported three dental rows reflect rows of primary, secondary and tertiary and replacement tooth germs.

More importantly, three observations in Shaw's (1979, 1982, 1986) cross-sectional studies of the development of the dentition in this animal are reconsidered. First, tooth formation does not start at the back of the jaw and advance toward the midline. Rather, tooth germ initiation begins abruptly in the mesial two-thirds of the jaw quadrant with the establishment of the IDR, and it then proceeds distally creating the DDR. Second, primary tooth germ development is not sequential from back to front. Though the sequential appearance of primaries is usually seen from front to back in the extending DDR, the order of initiation in the IDR is variable with no directionality. Finally, the appearance of the dental row is not a simple *ab initio* development between two alternating dental series, the even- and odd-numbered loci. Alternation is a common feature of the larval dentition, but it is secondary to the developmental dynamics of the primary, secondary and tertiary tooth germs.

The discrepancies between the results of this study and that of Shaw appear to underline the differences between the longitudinal and cross-sectional methods. In particular, the latter carries two important assumptions: (1) the external criteria by which animals are staged correlate to dental development; and (2) the pattern of tooth initiation is the same between different animals. This longtitudinal study demonstrates significant variability for both these factors. These results are consistent with Trueb and Hanken (1992) who conclude that skeletal development in this tadpole is more variable than previously reported and correlates poorly with external morphology.

Early dental initiation patterns in *X. laevis* larvae are not consistent with those predicted by any of the standard theories on the development of the dentition--Zahnreihen, Clone and New Progress Zone theories.

Edmund's (1960, 1962, 1969) Zahnreihen Theory suggests that tooth initiation is a function of a morphogen travelling from the front of the jaw to the back, inducing successive primordia at regular intervals. In the present study, not one dental initiation pattern in the 44 jaw quadrants was remotely similar to that predicted by Edmund. It is important to note that his theory was never based upon experimental evidence, and that Osborn (1971) and Westergaard and Ferguson (1986) demonstrated how Edmund skewed the embryological descriptions of Woerdeman (1919, 1921).

Osborn's (1971, 1973, 1978, 1984, 1993) Clone Theory proposes that a discrete group of ectomesenchymal cells gives rise to the dentition. The clone of cells grows in concert with jaw expansion and dental initiation is controlled intrinsically, occurring once the necessary conditions are met outside zones of inhibition surrounding developing teeth. However, in *X. laevis* larvae the length of the dental row far exceeded jaw growth, suggesting that it was not a *direct* response to jaw expansion. More specifically, during the initiation of the IDR, the average amount of quadrant jaw growth was only 71 µm (range: 0-200 µm) while the average IDR length was 1137 µm (range: 800-1500 µm). Significantly, Osborn's (1971) embryonic tooth initiation pattern in *L. vivipara* is strikingly similar to that in *X. laevis*. The first tooth germs that could "be recognized with certainty" appeared abruptly in a manner like those in the tadpole IDR. These were found in a jaw (labelled "Dentition C") which featured 6 and 7 tooth germs in the right and left jaw quadrants, respectively. These all appear to be at the same stage of development, and Osborn's attempt to deduce the actual initiation order is the result of forcefully extrapolating the back-to-front wave replacement phenomenon of the older dentitions upon the developing embryonic dentition.

Westergaard and Ferguson (1986, 1987, 1990) offered the New Progress Zone Model for the development of the dentition incorporating the view that dental patterning first resides in the oral epithelium (Mina and Kollar, 1987). This model features two aspects. First, an "early initiation stimulus" along with an inhibition mechanism "spread[s] in the epithelium". Second, this theory also envisions a proliferative epithelial "progress zone" that expands in concert with jaw growth, and that with either a cell lineage phenomenon and/or a positional information phenomenon epithelial cell division ceases, giving rise to new dental anlagen. However, the dental initiation patterns in this study are not consistent with an initiatory stimulus spreading out from the first odontogenic site. Specifically, the initiation patterns in the IDR are not sequential. Moreover, the large discrepancy between actual jaw growth and dental row development argues that the development of the dentition is not a simple and direct response to jaw growth.

The Zahnreihen, Clone and New Progress Zone theories were constructed from standard histological data on reptilian models, and the differences noted between these models and the development of the dentition in *X. laevis* may prove to be *sui generis* to the amphibian class.

Finally, experimental investigations support this descriptive study. The surgical removal at Stage 45 (6 Days Post-Fertilization; length: 10-11 mm) of the middle third of the jaw quadrant two weeks before the beginning of odontogenesis was performed and the defect maintained in order to interrupt purported traveling morphogens and/or growing clones/progress zones (Chapter 5). However, dental patterning in the unoperated mesial and distal thirds of the jaw quadrant proceeded in a fashion consistent with that of the IDR and DDR, respectively. In another experimental study, a primary tooth germ was extirpated resulting in the accelerated initiation of the adjacent tooth germs. This suggests that dental initiation is controlled locally and not through an overarching mechanism as proposed by the Zahnreihen theory (Chapter 4).

TABLE 2-1. TOOTH GERM DEVELOPMENT STAGES
LATE STAGE 54 TO STAGE 61

NAME	STAGE	ABREVIATION	DESCRIPTION
Partial Lacuna	1	(PL)	Barely discernible lacuna
Empty Lacuna	2	(EL)	Well-circumscribed lacuna 125-150 X 100 microns
Hint of a Tooth	3	(HT)	Well-circumscribed lacuna Conical tooth <25 microns
Small Tooth	4	(ST)	Well-circumscribed lacuna Conical tooth 25-50 microns
Medium Tooth	5	(MT)	Well-circumscribed lacuna Conical tooth 50-75 microns
Large Tooth	6	(LT)	Well-circumscribed lacuna Conical tooth 75+ microns

Table 2-1. Tooth Germ Development Stages in *Xenopus Laevis* between Late Stage 54 to Stage 61. The dissecting microscope with a graticule (25 µm units) was employed to record *in vivo* the appearance of the tooth germs and to measure their developing teeth.

TABLE 2-2. DEVELOPMENTAL TOOTH GERM TYPES & DEVELOPMENTAL TOOTH GERM CONFIGURATIONS:
22 ANIMALS AT STAGE 61

TYPE	INCIDENCE
Primary	391
Primary Twinned	10
Secondary Solitary	239
Secondary Doublet	10
Secondary Triad	48
Tertiary Doublet	10
Tertiary Triad	96
Mesial End	40
Replacement	59

CONFIGURATION	INCIDENCE
Solitary	239
Doublet	10
Triad	48
Null	55

Table 2-2. Developmental Tooth Germ Types and Developmental Tooth Germ Configurations in *Xenopus Laevis* at Stage 61. These classifications were based upon both *in vivo* germ tooth initiation patterns between late Stage 54 and Stage 61 as recorded under the dissecting microscope and histological analysis with the culture microscope at Stage 61 after the dental row was stripped from the suprarostral cartilage. Tooth germ types and configurations (or arrangement of tooth germs) reflect the time the tooth germs appeared in the mouth and their positions relative to each other. The final number of types and configurations was established at the end of the recording period at Stage 61.

Table 2-3. Tooth Germ Initiation Patterns. The dissecting microscope with a graticule (25 µm units) was employed to record the appearance and position of the tooth germs in the developing jaw quadrant. All measurements were adjusted to the most mesial tooth germ in the row (i.e., Tooth Position 1). Tooth germ development stages are in accordance with Table 2-1. 'Histological analysis' was done under the culture microscope. The 'alternation pattern' refers to the relative position of the adjacent tooth germs employing the following categories: L: labial; M: middle; P: palatal. Boxed tooth germs indicate primaries in the Initial Dental Row (IDR). Open rectangles indicate the triad dental configuration. DPF: days post-fertilization; ST: Nieuwkoop and Faber (1967) Stage; LT: length of tadpole in millimeters; JQL: jaw quadrant length in microns.

TABLE 2-3. TOOTH GERM INITIATION PATTERN. ANIMAL 1 (LEFT)

DPF	ST	LT	JQL																	
25	34	46	2150																	
26	34	49	2125												PL 1200					
27	34	49	2225				PL 350				PL 800				PL 1175					
28	34	49	2125		? 175		EL 400		EL 600		EL 800		EL 1000		EL 1175					
29	33	50	2150		EL 175		EL 350		EL 660		EL 850		EL 1050		EL 1175					
30	33	52	2225		EL 175		EL 350		EL 660		EL 800		EL 1025		EL 1225					
31	33	54	2275		EL 175		EL 400		EL 660		EL 825		EL 1025		EL 1250					
32	33	55	2375		EL 175		EL 375		EL 660		EL 825		EL 1025		EL 1250		? 1475			
33	35	56	2425		EL 175		EL 375		EL 575		HT800		EL 1025		EL 1250		EL 1450			
34	36	57	2425	PL 0	EL 175		HT 375		HT 575		ST 800		HT1025		EL 1200		EL 1450			
35	36	59	2500	EL 0	HT 150	PL 275	ST 350	PL 500	ST 600	PL 700	MT 800		HT 1025		HT 1250		EL 1500			
36	36	60	2475	EL 0	ST 125	EL 275	ST 375	EL 500	ST 575	EL 700	MT 825		ST 1050		ST 1250		EL 1500			
37	37	61	2475	EL 0	ST 125	EL 275	ST 375	EL 500	MT 600	EL 700	MT 825		ST 1050	PL 1150	ST 1275		EL 1525		EL 1750	
38	37	62	2450	EL 0	ST 125	EL 275	ST 375	EL 500	MT 600	EL 700	MT 825	EL 925	ST 1075	PL 1150	ST 1275		HT 1550		? 1750	
39	38	64	2475	EL 0	MT 100	EL 275	MT 375	EL 500	MT 575	EL 700	LT 850	EL 925	MT 1050	EL 1175	MT 1300		HT 1550		EL 1775	
40	39	64	2500	EL 0	LT 150	EL 275	LT 400	EL 500	LT 600	EL 700	LT 850	EL 925	MT 1050	EL 1175	MT 1300	EL 1425	ST 1550		EL 1775	
41	60	63	2500	HT 0	LT 150	EL 275	LT 400	EL 500	LT 650	EL 750	LT 850	EL 975	MT 1075	EL 1200	MT 1325	EL 1425	ST 1600		EL 1775	
42	60	63	2500	ST 0	LT 175	HT 275	LT 400	HT 525	LT 650	HT 775	LT 900	EL 1000	LT 1075	EL 1250	LT 1325	EL 1450	MT 1600		EL 1775	? 1950
43	61	64	2500	ST 0	LT 150	HT 275	LT 400	ST 525	LT 650	ST 775	LT 900	HT 1000	LT 1125	EL 1250	LT 1400	EL 1500	LT 1650		EL 1825	EL 1975
Histological Analysis				ST	LT	ST	LT	ST	LT	ST	LT	ST	LT	HT	LT	EL	LT	PL	HT	EL
Alternation Pattern				P	L	P	L	P	L	P	L	P	L	P	L	P	L	P	L	P
Replacement Teeth					PL															
Tooth Position				**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**

Figure 2-1. Dental Row Development in *Xenopus Laevis* between Late Stage 54 and Stage 61. This schematic representation of dental development in the upper jaw quadrant is based upon both *in vivo* germ tooth initiation patterns between late Stage 54 and Stage 61 as recorded under the dissecting microscope and histological analysis with the culture microscope at Stage 61 after the dental row was stripped from the suprarostral cartilage. The Initial Dental Row (IDR) appears abruptly in the mouth during the first 4 days of the development of the dentition (range: 4-8 tooth germs). The Distal Dental Row (DDR) then extends out from the IDR with the sequential addition of tooth germs over the next 2-3 weeks. The interdental spaces between these first tooth germs in both rows accommodate interdental tooth germs which develop during the recording period. The addition of one or two tooth germs at the mesial end of the IDR results in the IDR being renamed the Mesial Dental Row (MDR). By Stage 61, rapid and dramatic metamorphic change in the head ends the *in vivo* recording and the MDR and DDR make up the Complete Dental Row (CDR) which features between 17-23 tooth germs (average: 20.5). Jaw growth is drawn to scale and shows a 13.4% increase between late Stage 54 and Stage 61.

FIGURE 2-1. DENTAL ROW DEVELOPMENT IN XENOPUS LAEVIS
LATE STAGE 54 TO STAGE 61

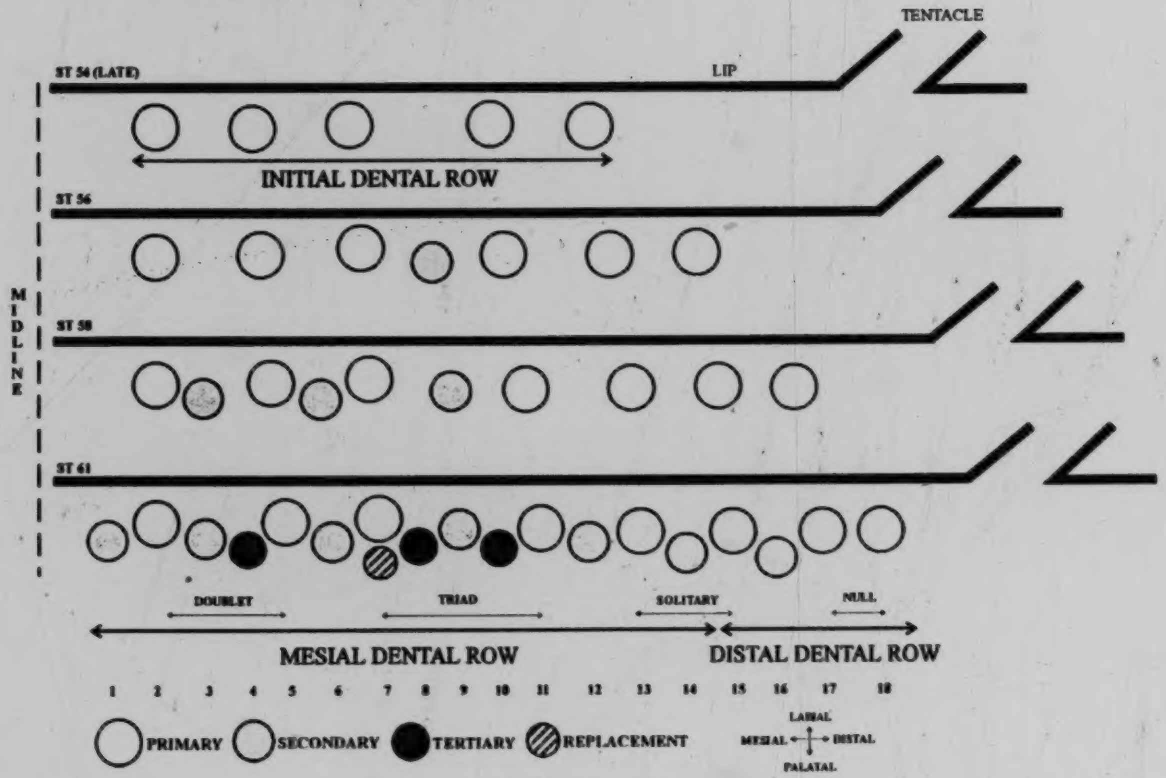

TENTACLE
ST 54 (LATE)
LIP
INITIAL DENTAL ROW
ST 56
ST 58
ST 61
MIDLINE
DOUBLET
TRIAD
SOLITARY
NULL
MESIAL DENTAL ROW
DISTAL DENTAL ROW
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
PRIMARY
SECONDARY
TERTIARY
REPLACEMENT
LABIAL
MESIAL
DISTAL
PALATAL

FIGURE 2-2
GENERAL TOOTH GERM DEVELOPMENT PATTERN
AVERAGE OCCUPANCY TIME OF TOOTH GERMS
AT TOOTH POSITION DURING ORP
LEFT & RIGHT: 44 JAW QUADRANTS

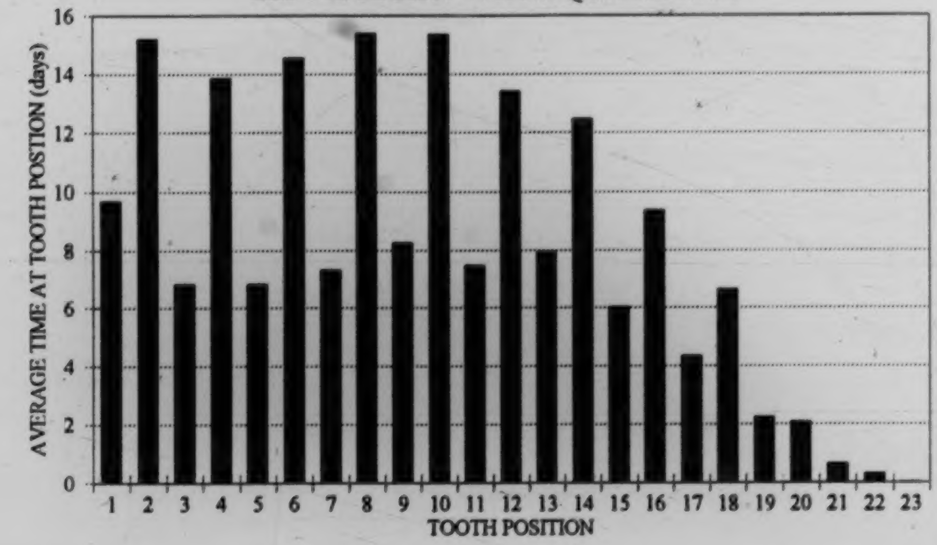


Figure 2-2. General Tooth Germ Development Pattern in *Xenopus Laevis* between Late Stage 54 and Stage 61. This composite shows the average number of days a tooth germ is present at every tooth position regardless of its developmental type during the odontogenic recording period (ORP). It shows a characteristic "plateau" of the even-numbered tooth germs in the mesial segment of the jaw quadrant and a "slope" of these tooth germs in the distal segment.

FIGURE 2-3
PRIMARY TOOTH GERM DEVELOPMENT PATTERN
AVERAGE OCCUPANCY TIME OF PRIMARIES DURING ORP
LEFT & RIGHT: 44 JAW QUADRANTS

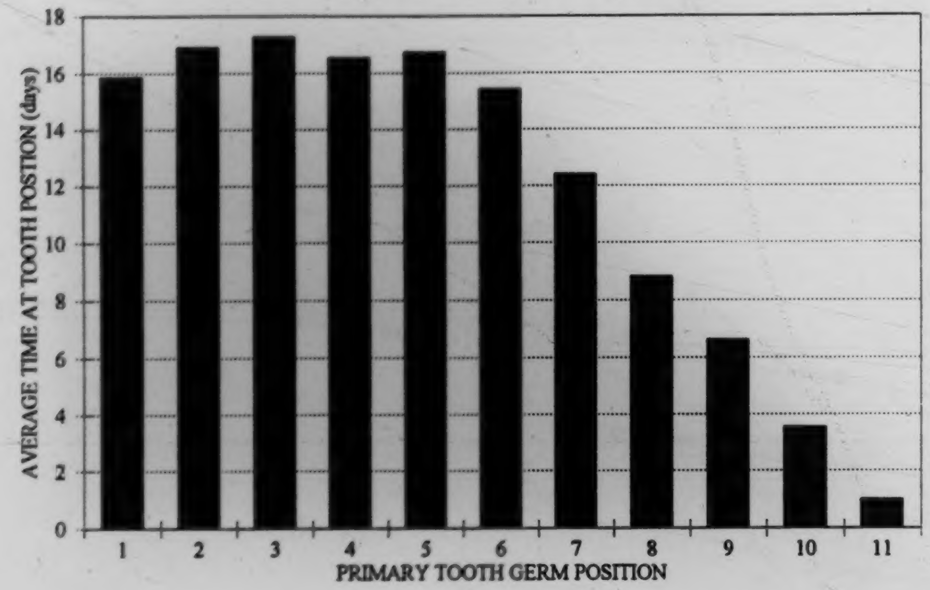


Figure 2-3. Primary Tooth Germ Development Pattern in *Xenopus Laevis* between Late Stage 54 and Stage 61. This composite shows the average number of days a primary tooth germ is present in the jaw quadrant during the odontogenic recording period (ORP). The position of the primaries in the quadrant is relative to only this tooth germ type and does not represent the final tooth position locus in the Complete Dental Row at Stage 61. The characteristic mesial "plateau" and distal "slope" pattern is evident with these tooth germs.

Figure 2-4. Tooth Germ Initiation Patterns in *Xenopus Laevis* between Late Stage 54 and Stage 61. Tooth germ initiation pattern variability is noted between four jaw quadrants. Four sequential triads are present in **A**. The IDR slopes toward the midline in **B**. The IDR in **C** extends 19 tooth positions and the DDR has only 3 tooth germs. Pattern variability is seen between the right and left sides of the same animal (**A** and **D**). The Initial Dental Row (IDR) appears abruptly in the mouth during the first 4 days of the development of the dentition and the primary tooth germs of this row are marked with an asterisk (*). The thick bar below tooth position numbers indicate the triad confirguration.

FIGURE 2-4. TOOTH GERM INITIATION PATTERNS:
OCCUPANCY TIME OF TOOTH GERM AT TOOTH POSITION DURING THE ORP

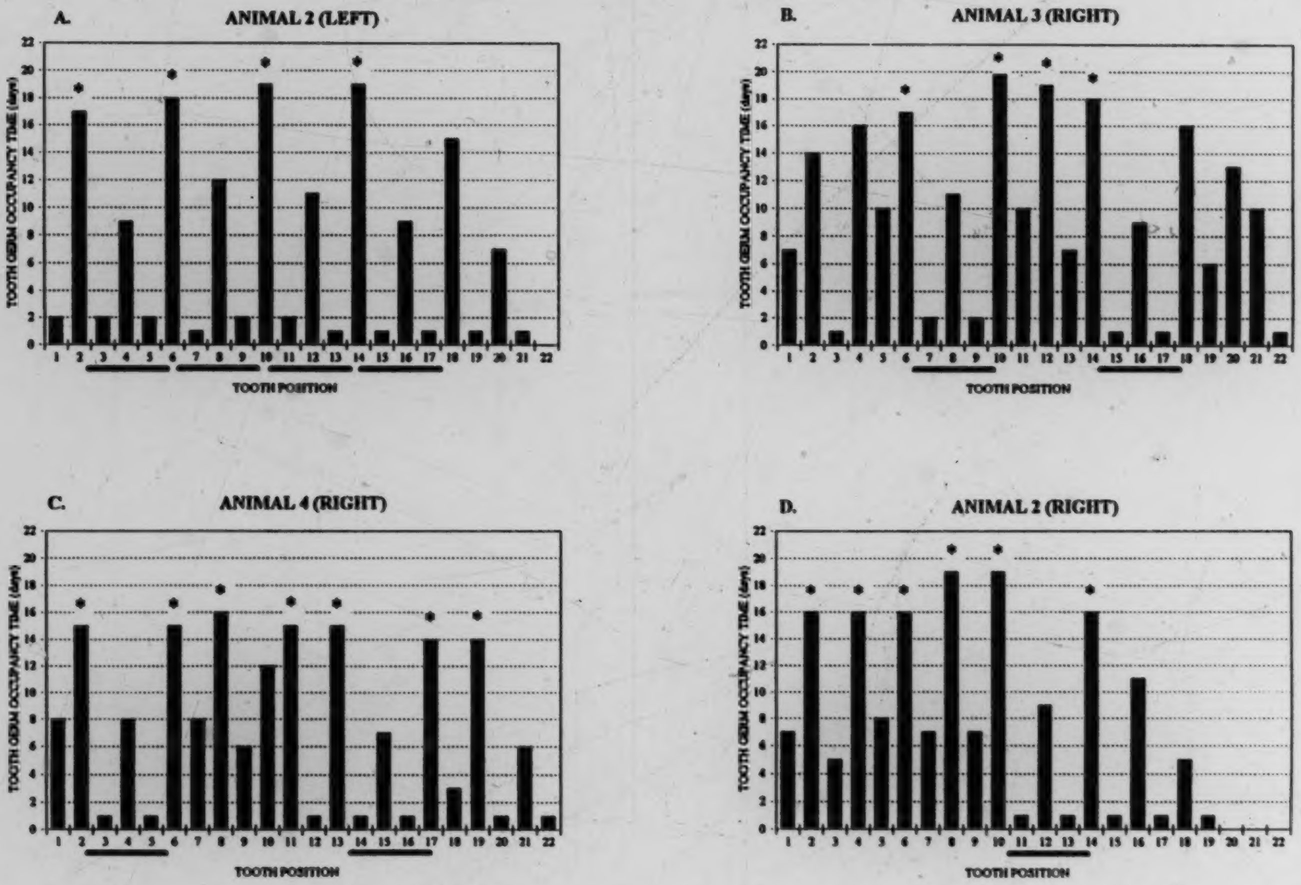


Figure 2-5. Initial Dental Row Tooth Germ Initiation Patterns in *Xenopus Laevis* between Late Stage 54 and early Stage 55. The dissecting microscope with a graticule (25 µm units) was employed to record *in vivo* the initiation of the primary tooth germs in the developing Initial Dental Row. These five tadpoles reveal variable initiation patterns across the jaw quadrant with coincident initiations and a lack of initiation order and direction. As a result, there is no definitive single site for the initiation of the dentition.

FIGURE 2-5. INITIAL DENTAL ROW: TOOTH GERM INITIATION PATTERNS

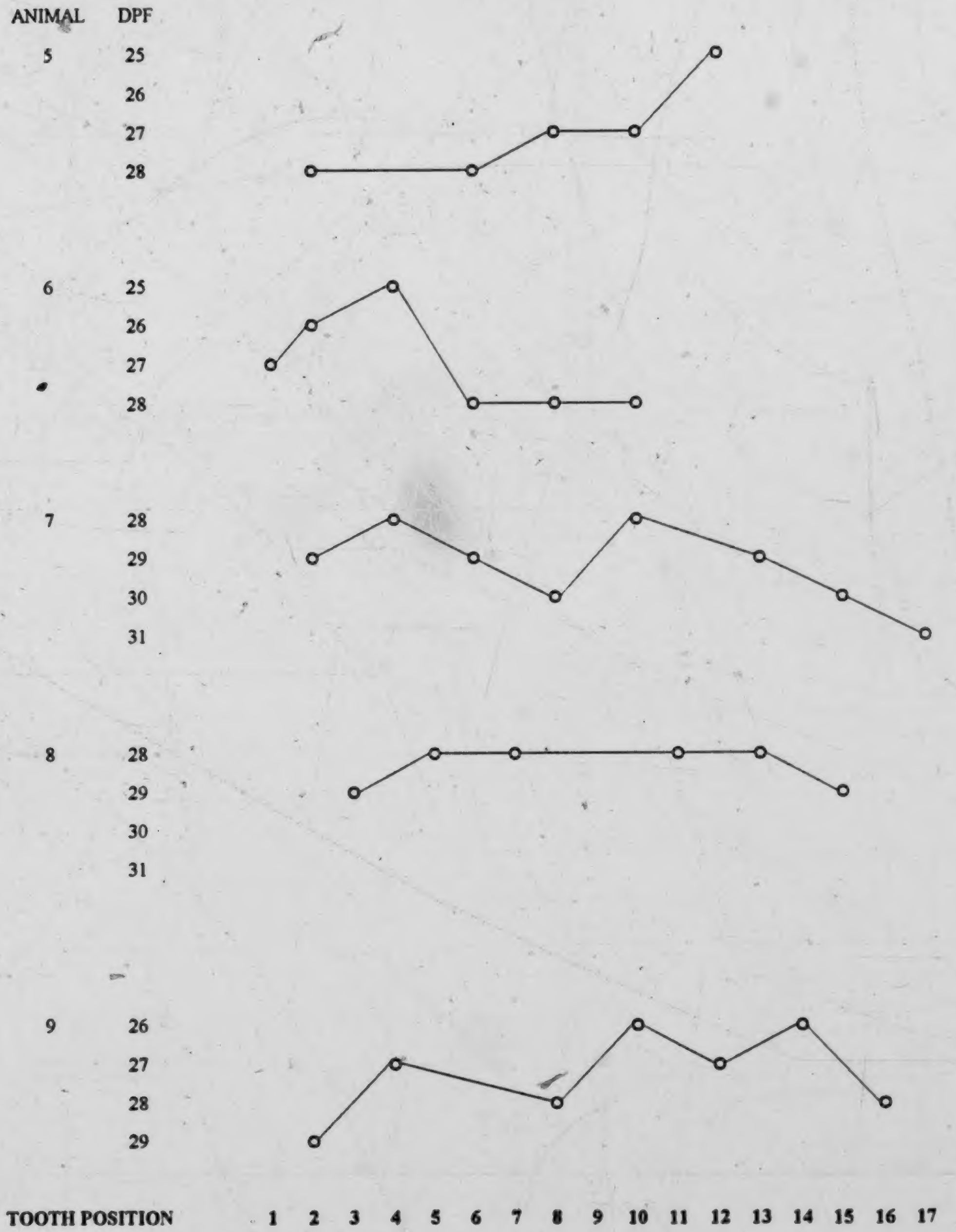


PLATE 2-1

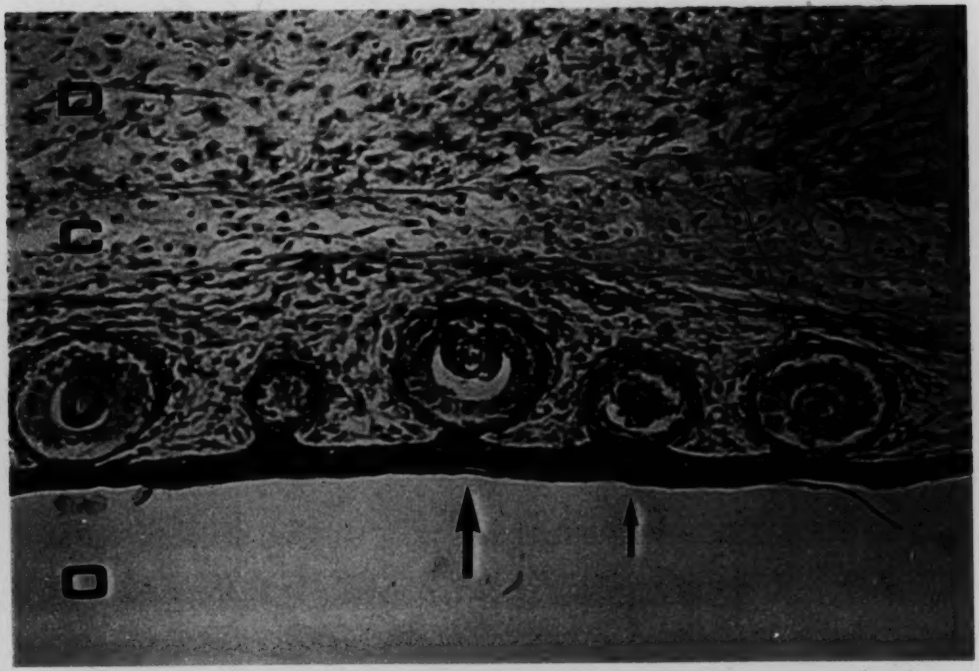


Plate 2-1. Transverse section of the upper jaw and dental row at Stage 59. Mature tooth germ with medium tooth (Stage 5 [MT]; long arrow). Incipient tooth germ (Stage 2 [EL]; short arrow). Dorsal mesenchyme (D). Suprarostral cartilage (C). Oral cavity (O). Stained with haematoxylin and eosin. Magnification (X 51).

Plate 2-2A (MESIAL SEGMENT). The Initial Dental Row (IDR) and extending Distal Dental Row (DDR) at late Stage 55. The dental row was stripped from the suprarostral cartilage and viewed under culture microscope. IDR tooth germs (thin closed arrows). DDR tooth germs (thick closed arrows). Partial lacuna of primary tooth germ (open arrow). Labial is toward the top of the page; distal and mesial are to the left and right page margins, respectively. Stained with methylene blue. Magnification (X 32).

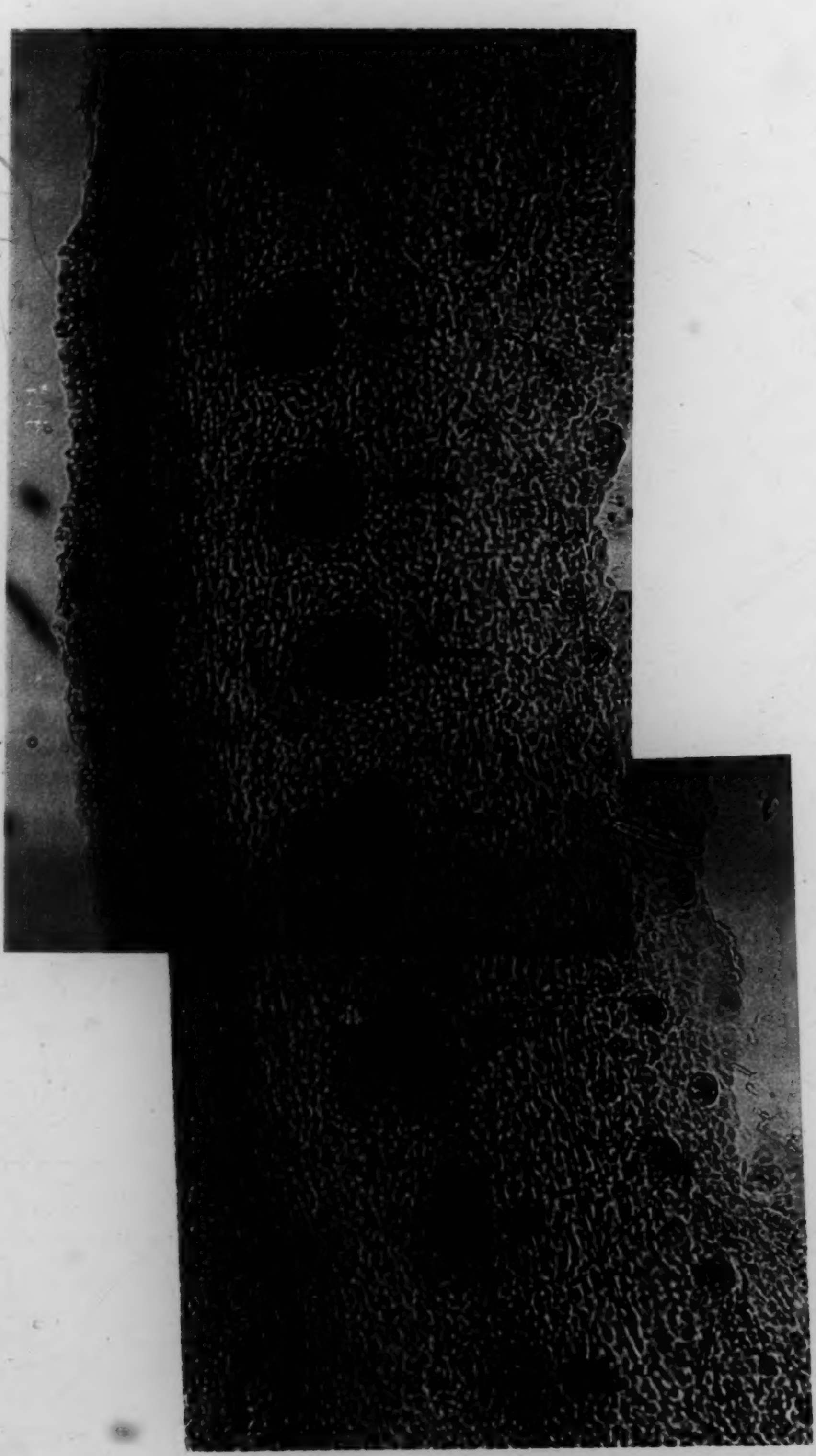


PLATE 2-2A (MESIAL SEGMENT)

Plate 2-2B (DISTAL SEGMENT). The Initial Dental Row (IDR) and extending Distal Dental Row (DDR) at late Stage 55. The dental row was stripped from the suprarostral cartilage and viewed under culture microscope. IDR tooth germs (thin closed arrows). DDR tooth germs (thick closed arrows). Tentacle (T). Labial is toward the top of the page; distal and mesial are to the left and right page margins, respectively. Stained with methylene blue. Magnification (X 32).

PLATE 2-2B (DISTAL SEGMENT)

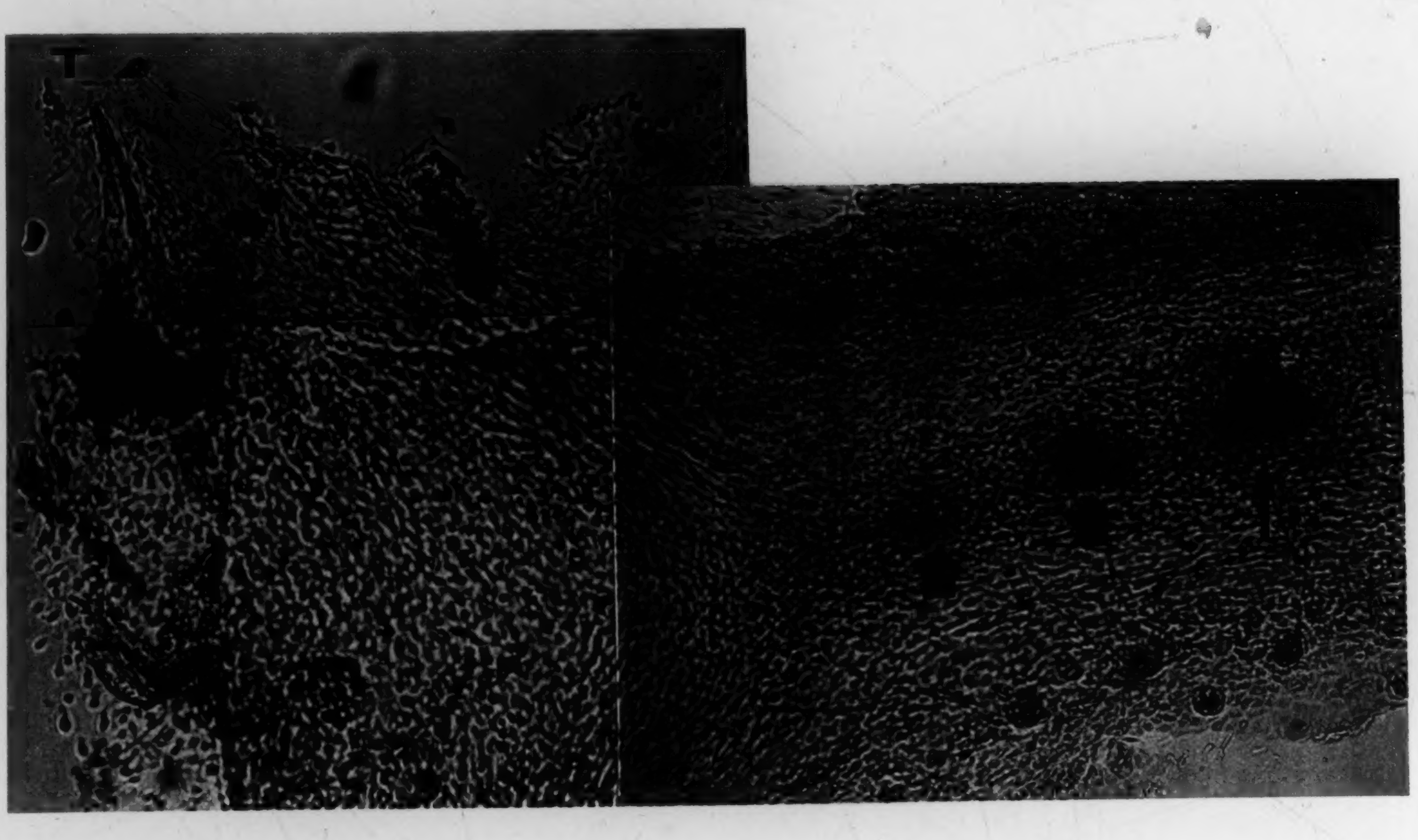


Plate 2-3A (MESIAL SEGMENT). The Complete Dental Row at Stage 61. The dental row was stripped from the suprarostral cartilage and viewed under culture microscope. Left tooth positions 1 to 8 and right tooth positions 1 to 3. Labial is toward the top of the page; distal and mesial are to the left and right page margins, respectively. Unstained. Magnification (X 32).

PLATE 2-3A (MESIAL SEGMENT)

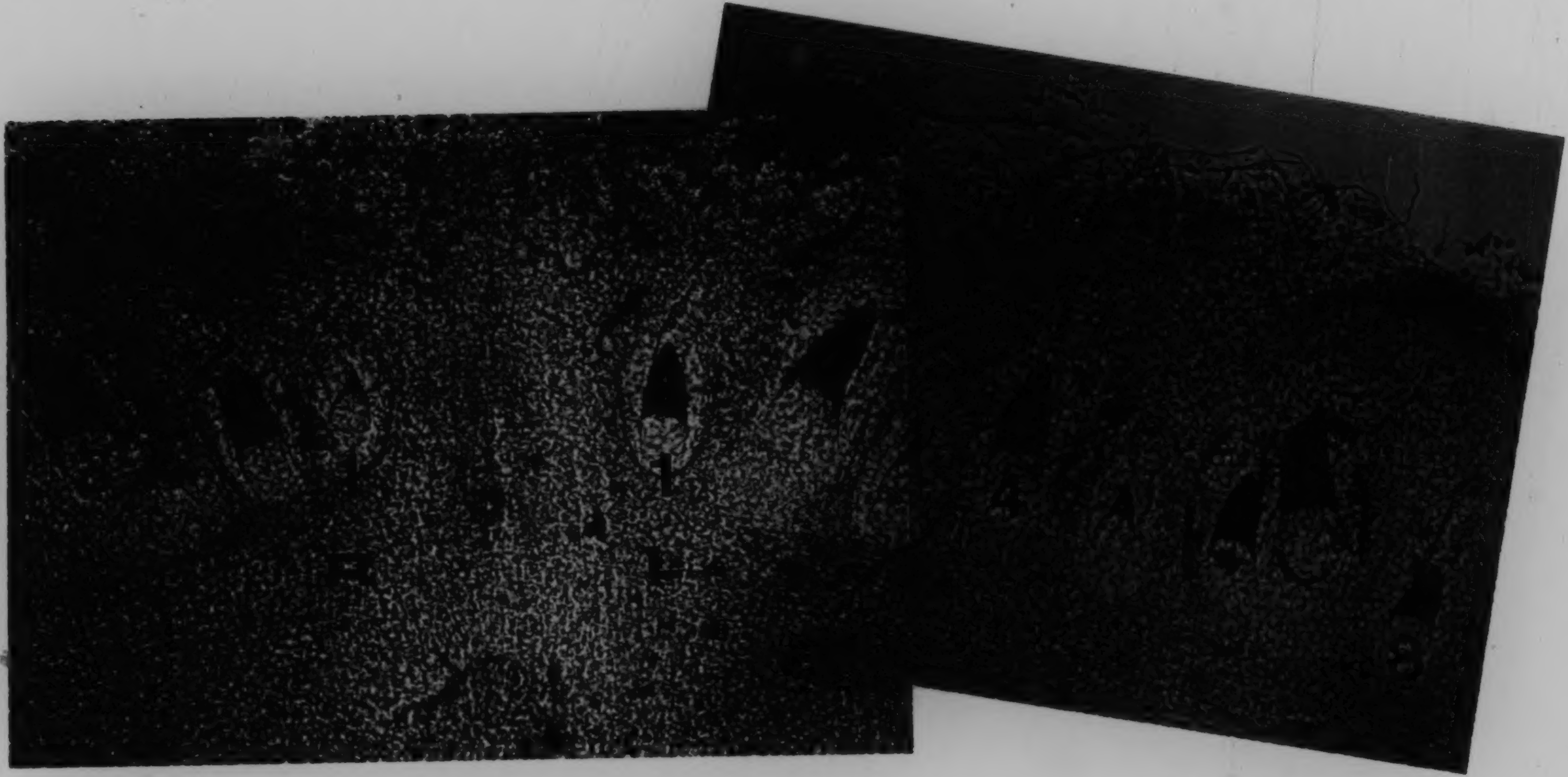


Plate 2-3B (DISTAL SEGMENT). The Complete Dental Row at Stage 61. The dental row was stripped from the suprarostral cartilage and viewed under culture microscope. Left tooth positions 8 to 18. Labial is toward the top of the page; distal and mesial are to the left and right page margins, respectively. Unstained. Magnification (X 32).

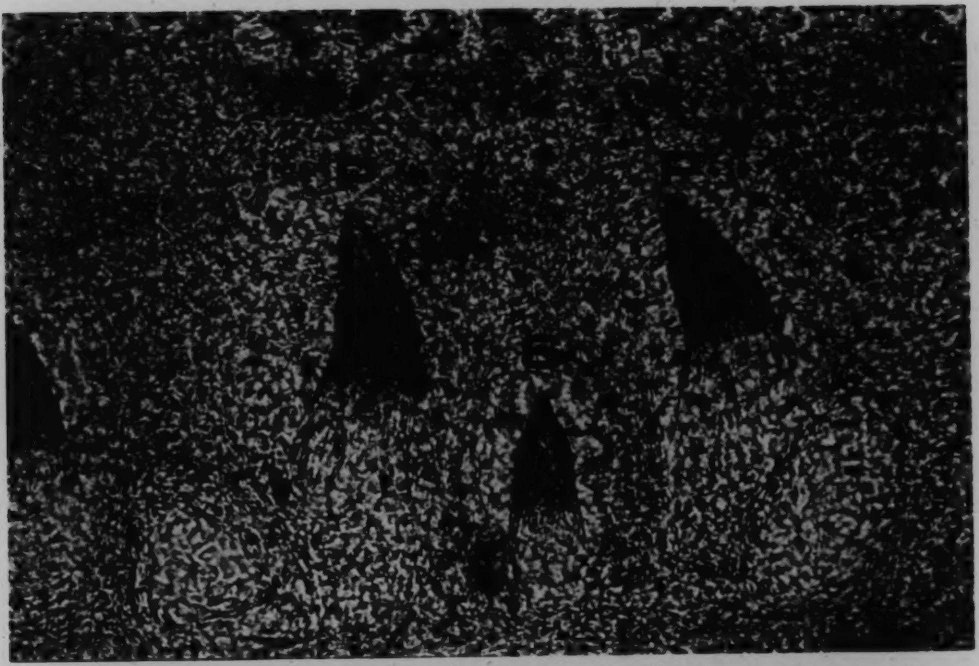
PLATE 2-3B (DISTAL SEGMENT)

Plate 2-4A. The Solitary Configuration at Stage 61. Primaries (P) and secondary (S). Labial is toward top margin of the page. Unstained. Magnification (X 51).

Plate 2-4B. The Triad Configuration at Stage 61. Primaries (P), secondary (S) and tertiaries (T). Labial is toward top margin of the page. Unstained. Magnification (X 51).

PLATE 2-4

A



B

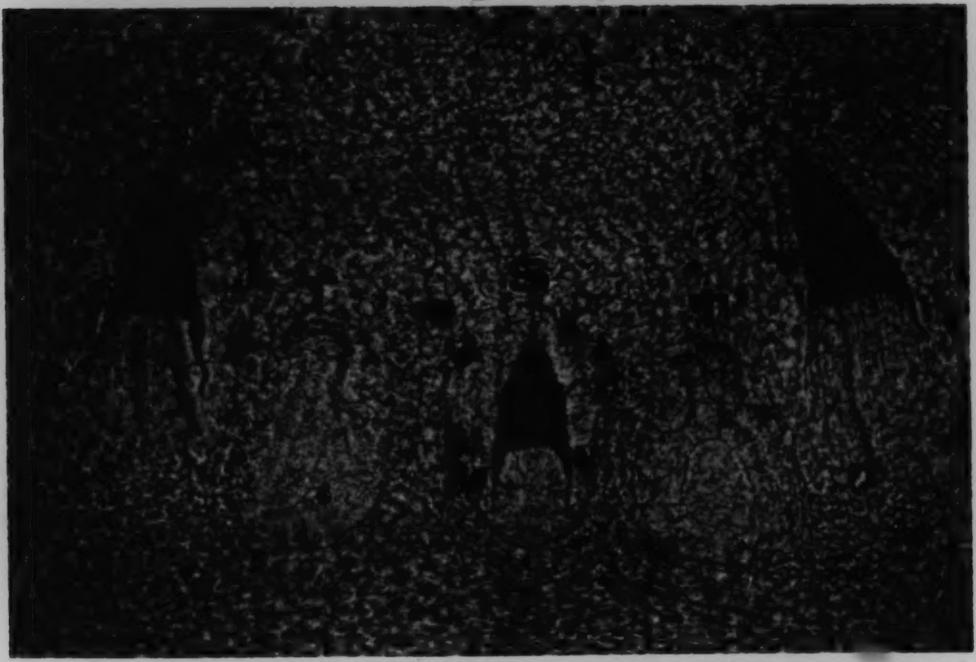


PLATE 2-5

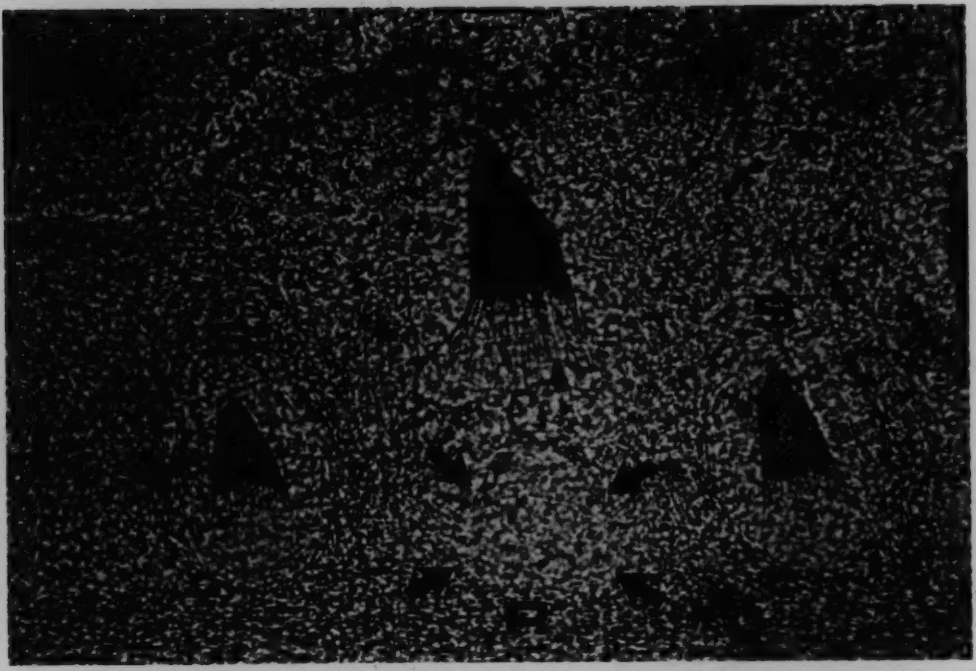


Plate 2-5. Replacement Tooth Germ at Stage 61. Primary (P), secondaries (S) and replacement (R; arrows mark tooth germ margins). Labial is toward top margin of the page. Unstained. Magnification (X 51).

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CHAPTER 3

DEVELOPMENTAL DYNAMICS IN THE EMBRYONIC DENTAL ROW: A LONGITUDINAL STUDY IN *XENOPUS LAEVIS* (DAUDIN) BETWEEN STAGES 54 AND 61

A. INTRODUCTION

The developing dentition offers a fruitful system for examining some of the mechanisms controlling organogenesis. Investigations of embryonic tooth initiation patterns in reptiles have been employed to formulate the most important theories on the development of the dentition. However, these cross-sectional studies fail to portray fully the dynamic nature of the developing dental row.

Edmund (1960, 1962, 1969) investigated the phenomenon of back-to-front tooth replacement at alternate loci in polyphyodont lower vertebrates. Employing both fossil and extant evidence and incorporating primarily the embryological descriptions of *Crocodylus porosus* (Woerdeman, 1919, 1921), he proposed that a morphogen released from a generator at the front of the jaw travels caudally, inducing successive primordia at regular spatial and temporal intervals. Edmund's Zahnreihen Theory gained popularity since it could describe the variety of wave replacement patterns in polyphyodonts (Goin and Hester, 1961; Cooper, 1963, 1965; Lawson, 1965, 1966; Lawson *et al.*, 1971).

Osborn (1970, 1971, 1972, 1973, 1974, 1975, 1977) responded to Edmund's model and over time developed the Clone Theory. First, he noted in the embryonic dentition of *Lacerta vivipara* that the earliest teeth in the mouth appeared with "varying frequency" at positions 3, 5, 6, 8, 10, and 13 (Osborn, 1971). This pattern was *contra* the orderly sequential initiation of tooth germs suggested by the Zahnreihen Theory. Second, Osborn deduced that dental initiation was from the back of the jaw to the front, challenging the front-to-back initiation pattern predicted by Edmund. Osborn (1978, 1984, 1993) later refined his model and suggested that "a discrete group of ectomesenchymal cells" expands in concert with the developing jaw and gives rise to the dentition. He also proposed that a sphere of inhibition surrounded the developing tooth, and that this not only ensured the even spacing of the teeth in the jaw, but also could account for the tooth wave replacement patterns. Osborn concluded that the entire dentition in the lower jaw quadrant of reptiles is the result of one clone of cells (termed "the clone determinant") initiated near its middle. The clone expands both anteriorly and posteriorly, giving rise to the first dental row. Interstitial growth between these first teeth then occurs, creating space for the development of interdental teeth in an "intervening dental row." In mammals, Osborn suggested that the dentition was composed of three clones--a posteriorly growing incisive clone, a single canine clone, and a molar clone which grows both posteriorly and anteriorly.

Westergaard and Ferguson (1986, 1987, 1990) noted that embryonic dental initiation patterns in the alligator *Alligator mississipiensis* were not consistent with the Zahnreihen Theory. In all jaw quadrants, the first tooth to appear develops at tooth position 3, and not at the anterior-most position as predicted by Edmund. Moreover, teeth are not initiated in strict sequence, but "interstitial teeth" develop between the "sites of primary tooth initiation." Westergaard and Ferguson also concluded that their results do not support the Clone Theory which they contended is based on the notion of regular alternation. Instead they proposed the New Progress Zone Model of dental development and incorporated recent dental embryology with its emphasis on the primacy of the oral epithelium in dental patterning (Mina and Kollar, 1987). Dental initiation begins in the epithelium at one site. From there an "early initiation stimulus" then spreads through the epithelium and is coupled with an "inhibition process," resulting in zones of competent epithelium capable of inducing the underlying mesenchyme into odontogenesis. This model also envisions a proliferative epithelial "progress zone" that grows in concert with the jaw growth, and that with either a cell lineage phenomenon and/or positional information phenomenon epithelial cell division ceases, giving rise to new dental anlagen. Moreover, interstitial growth between the first teeth opens regions for interdental development. Westergaard and Ferguson noted that new teeth develop at regular distances from older ones, suggesting the presence of an inhibitory zone calculated to be 300 μm.

Chapter 2 offered the first longitudinal record of the developing embryonic dentition. Dental initiation patterns in the larvae of the South African clawed frog *Xenopus laevis* were not consistent with the Zahnreihen, Clone or New Progress Zone theories on the development of the dentition. Regarding the first theory, the first teeth to develop in these tadpoles did not appear in a sequential front-to-back order, but their initiation was irregular followed by varying interstitial tooth development. Regarding the latter two theories, variable and coincidental initiations of the tooth germs occurred across the first dental row, and the initiation of these did not proceed sequentially away from the first dental locus to appear in the mouth. Moreover, the number of interdental teeth and their time of appearance was also variable. Finally, these two models are intimately related to jaw growth, but in the tadpole an initial dental row (IDR) of 4-8 tooth germs (average length: 1137 µm; range: 800-1500 µm) quickly developed over a few days with little to no corresponding jaw growth (average: 71 µm; range: 0-200 µm).

This study is a longitudinal investigation of the development of the early dentition in larval *X. laevis* (Daudin) which focusses upon the dynamic nature of the developing dental row. These results and those previously reported (Chapter 2) are employed to propose a model for the development of the embryonic dentition--the Odontogenic Field Theory.

B. MATERIALS AND METHODS

Animals were obtained by induced breeding with human chorionic gonadotropin (HCG, Sigma Chemical Co., St. Louis, MO).. At 4 PM adult females were injected with 800 IU of HCG and placed overnight with adult males which had received 400 IU of HCG at the same time. Embryos developed in gently aerated tap water treated with 1% sodium thiosulphate (2mL per liter) and maintained at room temperature. Once they were free-swimming tadpoles at 6-8 days post-fertilization, the animals were transferred to 40 L stock tanks (approximately 200 animals/tank) featuring a one way constant flow (20 L/hr) of filtered (by activated carbon) and dechlorinated (treated with sodium thiosulphate) water held at a constant pH (7.8) and constant temperature (20 ± 0.5 C°). The animals were fed Tadpole Powder (Nasco, Fort Atkinson, WI) sprinkled over the water surface daily at 7 AM and 6 PM. Two varieties of snails aided in maintaining the cleanliness of the tanks. Excess detritus was removed biweekly. A 12 h photo period was maintained (7 AM to 7 PM).

Twenty-four precocious tadpoles at Stage 53 (Nieuwkoop and Faber, 1967) were separated from the stock population and placed into groups of four in 10 L brown buckets that featured the above conditions. Small 1 mm incisions on the dorsal skin between the olfactory nerves served to identify the tadpoles. The animals were anesthetized daily with 2% aminobenzoic acid ethyl ester (MS 222, Sigma Chemical Co., St. Louis, MO), and their mouths were opened with the aid of a retractor fabricated from orthodontic ligature wire. The transparency of the tadpoles and the and the horizontal orientation of their developing tooth germs permits the longitudinal recording of the first 15-20 days of the development of the dentition, the Odontogenic Recording Period (ORP). The positions of the developing tooth germs were recorded using a graticule mounted on a binocular dissecting microscope. The position of the tooth germs in the developing dental row was measured from the tooth germ nearest the midline. The tooth germ at position 2 usually appeared early in development and often served as the reference point. All measurements were made from the center of the tooth germs. Recording began during mid-Stage 54 about 2-3 days before the first evidence of odontogenesis under the dissecting microscope. It continued until Stage 61 when dramatic metamorphic changes in head morphology and increased tissue opacity made accurate recording impossible. Finally, two animals died during the ORP, leaving 22 animals.

Animals were sacrificed by anesthetic overdose (MS 222) at the end of the ORP. A strip of oral tissue containing the left and right dental rows was dissected from the overlying suprarostral cartilage and examined under a tissue culture microscope to determine the final tooth count.

The width of the maxillary jaw epithelium was measured in another 24 animals. Four animals at six different stages were selected at mid-54, 55, mid-55, 56, 57 and 59 (Days Post-fertilization: 27, 30, 33, 36, 39, 42, respectively). The animals were sacrificed by anesthetic overdose. The tissue was fixed in formalin, demineralized with a formic acid and sodium citrate solution, sectioned at 10 μm and stained with haematoxylin and eosin. The measured width of the epithelium was adjusted by assuming a 30% shrinkage due to histological processing (Bancroft and Stevens, 1990).

The terminology employed in Chapter 2 to describe the development of the early dentition in larval *X. laevis* is used in this chapter. 'Primary tooth germs' are defined as the first tooth germs to appear in the dental row. Dental development begins quickly over 2 to 4 days during late Stage 54 in the medial two-thirds of the jaw quadrant with a row of 4 to 8 primaries (Initial Dental Row--IDR). These are followed by the distal and sequential addition of more primaries (Distal Dental Row--DDR) over the next 2-3 weeks. On the average, 1 or 2 tooth germs are added to the proximal end of the IDR resulting in the renaming of this row as the 'mesial dental row' (MDR). By Stage 61 the MDR and DDR together form the 'complete dental row' (CDR). In both the MDR and DDR, interdental tooth germs develop between the primaries in a more palatal position. A 'secondary tooth germ' develops between two primaries, and together these three tooth germs constitute the 'solitary configuration.' A 'tertiary tooth germ' appears between a secondary and a primary. The arrangement of a secondary and a tertiary between two primaries is a 'doublet.' A pair of tertiaries separated by a secondary and bordered by two primaries results in the 'triad configuration.' The 'null configuration' is a pair of primaries with no interdental tooth between them. [Note: The dental terms 'mesial' and 'distal' are employed in this paper to refer to the 'medial' and 'lateral' aspects of the dental row in each jaw quadrant, respectively (Bitgood and McMahon, 1995; Mina *et al.*, 1995). 'Buccal' means 'toward the lip' while 'palatal' indicates 'toward the palate.'].

Statistical analysis employed the Systat package.

C. RESULTS

(1) INTERDENTAL GROWTH

The distance between the primary tooth germs increased only slightly during the ORP (**Table 3-1**). The average growth at all sites between primaries in the CDR was 24 µm by Stage 61. The average interdental growth between CDR primaries from the time of their initiation to when their secondaries appeared was 20 µm.

(2) DENTAL ROW LENGTH AND TOOTH GERM NUMBER

The final length of the dental row correlated with the number of tooth germs in the MDR and DDR (r: 0.84 and 0.91, respectively). By Stage 61 the average interdental distance between tooth germs was similar across the entire dental row (MDR: 110 µm; DDR: 118 µm).

(3) INTERDENTAL DISTANCE AND TOOTH GERM NUMBER/DENTAL CONFIGURATION

Figure 3-1 plots the average distance between primary tooth germs in the CDR against the number of tooth germs that developed between them. The r value of 0.80 suggests that interdental tooth incidence, and ultimately dental configuration, related directly to the primary tooth germ interdental distance. In other words, wide interdental spaces developed more interdental teeth. Focussing only on IDR primaries and their secondaries offered a similar relationship (r: 0.75). **Table 3-1** reveals that the resultant dental configurations reflect the average interdental distance between primaries in the CDR during the ORP: null (114 µm), solitary (217 µm), doublet (281 µm) and triad (329 µm).

Similarly, in the IDR the average distance between primaries at their initiation related to the number of tertiaries that later developed in these dental rows (r: 0.76). That is, dental rows with wider primary interdental spaces accommodated more tertiaries, resulting in an increased incidence in doublet and triad dental configurations.

The relationship between primary tooth germ interdental distance and interdental tooth germ number/dental configuration was also reflected in the differences between the left and right MDRs. The average primary tooth germ interdental distance was 254 µm and 235 µm, respectively (**Table 3-1**). On the left side 26.9% of the interdental distances between the primaries were greater than 300 µm compared to 14.0% of these on the right side. As a result, the left MDR primary interdental sites had on the average 1 more tooth germ for every 4 sites. More specifically, this dental row on the left side featured 25 triads and 4 doublets while the right had 15 triads and 1 doublet. Thus, the number of tertiary tooth germs in the left MDR was nearly double (54 to 31) that of the right MDR.

(4) INTERDENTAL DISTANCE AND DEVELOPMENTAL TIME

Figure 3-2 plots the average distance between any pair of adjacent tooth germs in the CDR that later developed one or more interdental tooth germ(s) against the time difference between the establishment of the pair of bordering tooth germs and the identification of the first interdental tooth germ (termed the 'interdental development time'). Interdental tooth germs discovered at the end of the ORP under the culture microscope were not included in order to avoid introducing a temporal distortion. An r value of -0.70 suggests that it took longer for an interdental tooth germ to develop between two closely positioned tooth germs. Focussing only on the developmental time of secondary tooth germs between primaries in the IDR resulted in a correlation of -0.75.

The relationship between the interdental distance and the development time of an interdental tooth is described by the following simple regression equation ($P < 0.0001$):

$$\textit{Interdental Development Time} = 14.8 - (0.73 \bullet \textit{Interdental Distance})$$

Verification of this regression was done with a one-way analysis of variance. Differing rates of development have previously been noted in this model system (Trueb and Hanken, 1992), and as a result it was necessary to determine whether the variable of time required standardization. A multiple factor regression test included: (1) the interdental distance ($P < 0.0001$), (2) the day post-fertilization that the first tooth appeared in the mouth ($P = 0.123$), (3) the last day post-fertilization of the ORP ($P = 0.557$), (4) the interdental initiation day post-fertilization ($P = 0.006$), and (5) the Nieuwkoop and Faber Stage ($P = 0.551$). As a result, it was determined that the time recorded in this study did not require standardization, and that factors 2, 3 and 5 could be deleted. The regression equation then becomes ($P < 0.0001$):

$$\textit{Interdental Development Time} = 14.33 - (0.48 \bullet \textit{Interdental Distance}) + (0.31 \bullet \textit{Interdental Initiation Day Post Fertilization}).$$

(5) INTERDENTAL SITE: DISTANCE, TOOTH TYPE AND DEVELOPMENT TIME

The interdental sites in the dental row featured a relationship between distance, tooth type and development time. **Figure 3-3** plots the average interdental distance for solitaries, doublets and triads in the CDR against the average interdental development time for their respective secondary tooth germ.

(6) DIFFERENCES IN THE DEVELOPMENT OF THE DENTITION BETWEEN THE IDR/MDR AND THE DDR

Differences in the manifestation of the developing dentition were seen between the IDR/MDR and the DDR. First, the average primary tooth germ interdental distance at initiation was 242 µm in the quickly established IDR, while it was 176 µm in the slowly extending DDR (**Table 3-1**). More specifically, the interdental distance in the DDR was found to decrease over time (r: -0.69). That is, DDR primaries initiated early in the ORP were further from their neighboring mesial primary than those that appeared later. Second, the range of distances between primary tooth germs at initiation was greater in the IDR (325 µm; range: 125-450 µm) than in the DDR (250 µm; range: 75-325 µm). Finally, the incidence of tertiary tooth germs in the IDR was about twice that of the DDR. On the average one tertiary was present for every 588 µm of IDR, while one appeared in every 1322 µm of DDR.

Despite these differences between the IDR/MDR and DDR, the average distance between the tooth germs was the same at the end of the ORP. The development of more interdental tooth germs in the IDR/MDR compensated for the wider initiation interdental distance between its primaries so that the average interdental distance by Stage 61 was nearly the same in both dental rows.

(7) MAXILLARY ORAL EPITHELIUM WIDTH DURING THE ORP

The average width of the maxillary oral epithelium in the jaw quadrant increased regularly during the ORP. A relationship emerged between epithelial thickness and the development stage/age of the animal (**Figure 3-4**).

D. DISCUSSION

Descriptive studies are helpful guides in the discovery of biological mechanisms despite their limits in determining the details of these processes. This study in conjunction with previous work on the developing dentition in *X. laevis* (Chapter 2) provide preliminary evidence to outline a model for the development of the dentition--the Odontogenic Field Theory.

The rapid establishment of the IDR argues that the initiation of the primary tooth germs is not a *direct* response to jaw growth since quadrant expansion is negligible during this time. Moreover, the non-sequential and variable appearance of tooth germs in the IDR suggests that dental initiation is not due to an initiatory molecular signal travelling the length of the jaw or to a clone of cells (either ectomesenchymal or epithelial) rapidly growing in a channel along the jaw. Instead, it appears that an Odontogenic Field (OF) becomes competent/established during late Stage 54 in *X. laevis*, and that it is within the OF that odontogenesis proceeds. Other descriptive evidence points to the possible developmental dynamic in the OF:

(1) The spacing between the primary tooth germs suggests the existence of Initiation

Zones (IZ) *prior* to dental morphogenesis. More specifically, once the necessary conditions for dental initiation are met in the OF, an IZ becomes established and tooth germ development then proceeds at the center of it. Two observations support this contention.

First, and most importantly, tooth germ clusters (i.e., the sequential arrangement of the null configuration) were not found in the CDR. In this row the null dental configuration rarely (3.4 %) appeared and there was not one example of two adjacent nulls in the 44 jaw quadrants. Moreover, the null configuration was not manifested in the IDR/MDR. That is, with the rapid and often coincidental appearance of tooth germs as seen particularly in the IDR, there must exist a mechanism that keeps them separated a certain distance from one another before their morphogenesis begins. If this were not the case, then the dental row would feature more null dental configurations, and tooth germ clusters would emerge. It appears then that the IZ acts like the long theorized "dental inhibitory zone" in that no other teeth are initiated within its boundaries while active (Gillette, 1955; Osborn 1971, 1978, 1984, 1993; Lumsden, 1978, 1979; Westergaard and Ferguson, 1986, 1987, 1990).

Second, the doublet dental configuration rarely occurred in the CDR (3.2%). For such a configuration to manifest, the secondary tooth germ must be skewed away from the middle of the interdental site to one side in order to create space for the development of the tertiary tooth germ. In contrast, secondaries usually develop midway between the bordering primaries as seen in the solitary and triad configurations. Similarly, previous theories have predicted that dental initiation occurs at a midpoint in the preodontogenic tissue (Gillette, 1955; Osborn, 1971, 1977, 1978).

(2) Variability marks the initiation patterns of the primary tooth germs in the IDR and points to the locally autonomous nature of a dental initiation mechanism in the OF (Chapter 2). More specifically, it appears that once the OF became competent during late Stage 54, IZs were established randomly and competitively. As a result, the pattern of the primary tooth germs reflects the sorting and ordering of the IZs at this early point in dental development. With such an autonomous mechanism it is reasonable to assume the existence of gaps of uncommitted OZ tissue that are variable in width between the IZs. Those IZs with significant gaps of uncommitted OZ tissue between them became doublet and triad dental configurations. Most IZs were positioned closely together and developed into the solitary configuration or at rare times the null configuration.

(3) The firm correlation between the interdental distance and the interdental development time is consistent with an odontogenic mechanism that is intrinsically controlled at the site of initiation. That is, initiation proceeded once the necessary conditions were met irrespective of the position of an interdental tooth in the dental row.

(4) The positions of the interdental and replacement tooth germs relative to their neighbouring primaries point to two more features of the developmental dynamic of the dental row. First, since growth does not account for it, there must be a recession of the theorized IZs associated with the primaries, otherwise the interdental tooth germs would have been positioned below the palatal margins of the primaries (**Figure 3-5A**). Instead, the interdental tooth germs appeared between the primaries and their buccal margins were found in a region between the buccal and middle thirds of the primaries, and the

replacement tooth germs were closely positioned to their primary (**Figure 3-5B**). The standard model for dental inhibitory zones proposes that interstitial grow between the primaries makes space for the interdental teeth (Osborn, 1971, 1977, 1978; Lumsden, 1978, 1979). A near doubling in the width of the interdental space is reflected in Osborn's (1977) diagram at the time the interdental tooth is initiated. This model also suggests that dental initiation begins at the margins of the inhibitory zone once the preodontogenic tissue "escapes" the inhibitory influence. In other words, initiation occurs in the "cracks" between the inhibitory zones with a small volume of preodontogenic tissue (**Figure 3-5C**). However, interdental growth between the primaries in *X. laevis* was negligible (5.5% increase in width at the IDR interdental sites). Furthermore, the lack of tooth germ clusters in this animal argues that a significant volume of preodontogenic tissue is required for dental initiation and that initiation does not begin in narrow spaces between inhibitory zones.

Second, the OF must extend palatally (either through growth or tissue conversion) during the ORP, opening up new areas for dental development. Interdental teeth were positioned progressively more palatally over time, and replacement teeth appeared directly palatal to their primary only at the end of the recording period . As a result, interdental tooth germs in narrow sites between the primaries were positioned quite palatally and they appeared late in the ORP because the necessary conditions for dental initiation were only met once the OF had extended palatally. These findings further suggest that dental initiation was autonomous and intrinsic, being dependent upon the conditions at the site of initiation.

(5) During the course of the ORP the average primary tooth germ interdental distance in the DDR decreased at a regular rate. Through this same period, the average width of the dental epithelium increased in a uniform manner. These observations are consistent with the notion that once a critical mass of OF epithelium was established dental initiation began. That is, with a thickening OF epithelium the critical epithelial volume for initiation was manifested within a narrower mesial-distal length of OF epithelium. As a consequence, DDR primaries were added nearer their adjacent mesial primary over time. Recent dental embryology in the mouse is consistent with this thesis. Mina and Kollar (1987), Kollar and Mina (1991) and Chapter 6 demonstrated that the first evidence of dental patterning resides in the oral epithelium.

Application of the Odontogenic Field Theory sheds light on the developmental dynamic of the dentition in the IDR/MDR and the DDR.

The distinct feature of the IDR is the rapid establishment of its tooth germs. Since these appear nearly simultaneously, it is reasonable to assume that the dental pattern of this row reflects a competition between multiple IZs prior to dental morphogenesis. The average interdental distance between these primaries at initiation was 242 µm, suggesting that the average width of the IZ would be near this distance. However, it is also reasonable to assume that this competitive process would produce gaps of free OF tissue between some of the IZs, and that this situation probably reflects the developmental dynamic in the doublet and triad dental configurations. As a consequence, the average initiation interdental distance between primaries of the solitary configuration most likely offers a truer average width of the IZ (218 µm) than the average of all the IDR interdental sites (242 μm). That is, the solitary configuration probably represents the edge-to-edge butting of the primary IZ margins when they were first established. This contention is further supported by the fact that solitaries make up 78% of the dental configurations in the IDR. As a result, with the average IZ determined to be about 218 μm, and the average tooth germ width at initiation about 100 μm, there was thus an average 59 μm border of IZ tissue on either side of the tooth germ when it appeared **(Figure 3-6)**. These measurements offer insights into the development of the triad and solitary dental configurations.

In the case of the triads in the IDR/MDR, the average interdental distance between the primary tooth germs at their initiation was 334 μm **(Table 3-1)**. Assuming the 59 μm border of IZ tissue adjacent to an initiated tooth germ, then the average triad featured about 116 μm of uncommitted OF tissue between the IZs of its primaries at initiation (**Figure 3-7A** and **B**). By the time the secondary tooth germ appeared, interstitial growth separated these primaries by 10 μm. As a result, the uncommitted tissue between the IZs, in combination with tissue released by the receding IZ, tissue gained through interstitial growth, and preodontogenic tissue produced by the palatally extending OF created the necessary conditions for another dental initiation. Thus, with more (116 μm + 10 μm + 59 μm + 59 μm = 244 μm) than the required (218 μm) IZ width of tissue readily available (**Figure 3-7C**), the triad secondary tooth germ appeared quickly (average: 5.2 days) and its buccal margin was slightly palatal to that same margin in the adjacent primaries (**Figure 3-7D**).

As previously suggested, the solitary dental configuration in the IDR/MDR had little if any uncommitted OF tissue between the IZs of its primary tooth germs. Even with the full recession of the IZs and the interstitial growth between the primaries by the time the secondary appeared (14 μm), there remained only a 132 μm interdental space between the primaries (**Figure 3-8B**). As a result, the critical mass of epithelial cells necessary for odontogenesis to proceed had to come from the thickening OF epithelium and the palatally extending OF. The solitary secondary tooth germ then appeared both later (average: 8.4 days) in the ORP and was positioned more palatally with its buccal margin at the level of the middle third of the adjacent primaries (**Figure 3-8C**).

The Odontogenic Field Theory may also explain dental development patterns in the DDR. First, the average interdental distance between DDR primaries of the solitary configuration is less than that in the IDR (170 μm and 218 μm, respectively. **Table 3-1**). A widening OF epithelium during the time when DDR primaries are initiated might account for this reduction in primary tooth germ interdental distance since a significant contribution to the critical epithelial volume necessary for initiation could come from that dimension (**Figure 3-9B**). Second, in contrast to the IDR where the OF was established rapidly across the jaw and IZs developed competitively, IZs in the DDR were established apparently in concert with the slow extension of the OF distally and the gradual thickening OF epithelium. As a result, odontogenesis in the DDR was more efficient than in the IDR and this row had half as many doublet and triad dental configurations (Chapter 2). That is, since the DDR featured fewer gaps of uncommitted or free OF tissue between its primary tooth germs than the IDR, there was less OF tissue available for tertiary tooth germ development.

Oster and Murray (1989) noted that most models for embryonic pattern formation are built on the notions of local activation and lateral inhibition. The prototypical model they construct employing the vertebrate limb is remarkably similar to the Odontogenic Field Theory. Three notable features include:

(1) Most biological patterns originate locally in an "organizing tissue," and from there they develop sequentially (eg., pteryla in birds; Sengel, 1976). Similarly, primary tooth germs in the tadpole dentition develop in the IDR and then the dental row extends distally with the sequential addition of more primaries. Further, Oster and Murray noted that when patterns grow simultaneously across a field they are less reproducible, a situation similar to the variability seen in the initiation patterns of the IDR primaries (Chapter 2). More specifically, Murray (1981a, 1981b) concluded that the complex patterns in the animal coat reflects the initial conditions of the developmental field. He added that since the initial conditions were dependent on the inherent stochastic effects of the developing system, they were unique to each animal as was the final pattern. Correspondingly, stochastic effects in the quickly activated tadpole odontogenic field during late Stage 54 in the medial two-thirds of the jaw quadrant can also account for the variety found between the primary tooth germ initiation patterns in the IDR.

(2) The activation or initiation of some embryonic structures is autocatalytic, being locally controlled, competitive, and dependent on a "sufficient tissue volume" or "critical domain size." More specifically, developmental foci are defined by "cell recruitment zones." This dynamic corresponds to a competitive establishment of dental initiation

zones in the tadpole odontogenic field. Once a critical epithelial volume is established, dental morphogenesis proceeds independently and is not influenced by distant regions of the developing jaw and dental row.

(3) Lateral inhibition creates a "zone of influence" around an embryological structure and this zone precludes the establishment of other embryonic foci within it. In tadpole tooth development, a related developmental dynamic is postulated to exist as no other dental initiations are manifested in the IZ until it has receded.

In sum, the Odontogenic Field Theory is a critical mass theory. Odontogenesis begins once a critical amount of OF epithelium is established. This model bears important resemblances to previous theories of the development of the dentition--Gillette (1955), Osborn (1971, 1978, 1993), Westergaard (1988a, 1988b), Westergaard and Ferguson (1986, 1987, 1990). Dental initiation is intrinsically controlled at the site of initiation and is independent of extrinsic factors from distant regions of the developing jaw and dental row. Moreover, an inhibitory/restrictive zone is associated with odontogenesis. Finally, the dental initiation pattern reflects the interaction between the conditions of initiation and inhibition. However, three features distinguish the Odontogenic Field Theory from the previous models. First, the development of the dentition is not *directly* related to jaw growth. Similarly, the amount of interstitial growth between the primary tooth germs is insignificant and does not seem to be an important factor in dental initiation. Second, the inhibitory/restrictive zone is established prior to actual dental morphogenesis. More specifically, an initiation zone is defined in the OF epithelium and a tooth germ later develops in its center. Third, the inhibitory effect of IZ is transitory. As the IZ later recedes, uncommitted OF epithelium is made available for the establishment of new IZs.

Table 3-1. Average Interdental Distances Between Primary Tooth Germs in *Xenopus Laevis* between Late Stage 54 and Stage 61. Interdental distances were measured *in vivo* under a dissecting microscope with a graticule (25 µm units). All distances were measured from the middle of primary tooth germs and divided into two groups. **A**. Measurements between primaries were made at *specific times*: (1) at the initiation of adjacent primaries, (2) at the initiation of the secondary tooth germ that appeared between adjacent primaries, and (3) at the end of the recording period (Stage 61) between adjacent primaries. **B**. The average interdental distance between primaries *during the ORP* was also examined in different segments of the dental row (IDR, DDR, MDR, CDR) for the developmental tooth germ configuration types (Null, Solitary, Doublet, Triad).

TABLE 3-1. PRIMARY TOOTH GERMS: AVERAGE INTERDENTAL DISTANCE

	AVERAGE INTERDENTAL DISTANCE (microns)	STANDARD DEVIATION (microns)	RANGE (microns)
A. AT SPECIFIC TIMES			
CDR–ALL CONFIGURATIONS			
INITIATION OF PRIMARIES	224	60	100-450
INITIATION OF SECONDARY	244	59	150-500
FINAL	248	64	150-575
IDR–ALL CONFIGURATIONS			
INITIATION OF PRIMARIES	242	66	125-450
RIGHT	235	64	125-425
LEFT	254	57	125-450
INITIATION OF SECONDARY	255	58	150-500
FINAL	263	64	150-575
IDR–SOLITARY CONFIGURATION			
INITIATION OF PRIMARIES	218	48	125-325
INITIATION OF SECONDARY	232	47	150-400
FINAL	238	52	150-450
IDR–TRIAD CONFIGURATION			
INITIATION OF PRIMARIES	334	52	250-450
INITIATION OF SECONDARY	344	42	250-525
FINAL	359	42	325-550
DDR–ALL CONFIGURATIONS			
INITIATION OF PRIMARIES	176	70	75-300
INITIATION OF SECONDARY	201	61	125-350
FINAL	212	52	150-400
DDR–SOLITARY CONFIGURATION			
INITIATION OF PRIMARIES	170	57	250-375
INITIATION OF SECONDARY	191	59	250-450
FINAL	200	62	300-500
B. DURING ORP			
CDR			
NULL	114	15	100-125
SOLITARY	217	41	125-325
DOUBLET	281	25	250-325
TRIAD	329	43	250-450
MDR			
NULL	none	N/A	N/A
SOLITARY	221	41	125-325
DOUBLET	291	25	250-325
TRIAD	334	43	250-450
DDR			
NULL	114	15	100-125
SOLITARY	207	38	125-300
DOUBLET	266	20	250-300
TRIAD	303	37	250-350

FIGURE 3-1. COMPLETE DENTAL ROW:
PRIMARY TOOTH GERM INTERDENTAL DISTANCE VS NUMBER OF INTERDENTAL TOOTH GERMS
LEFT & RIGHT: 44 JAW QUADRANTS

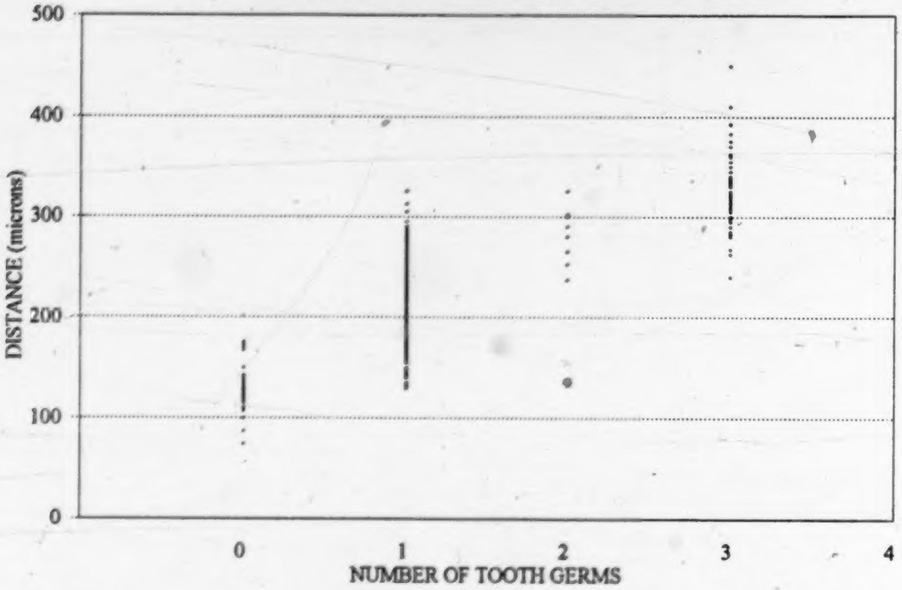


Figure 3-1. The average distance between adjacent primary tooth germs in the Complete Dental Row (CDR) relates directly with the number of tooth germs that developed between them (r: 0.80). Interdental distance is the average distance between the primaries during the recording period (late Stage 54 to Stage 61) as recorded *in vivo* with the dissecting microscope. The final number of tooth germs between any two primaries was determined by stripping the dental row from the suprarostral cartilage and examination under the culture microscope.

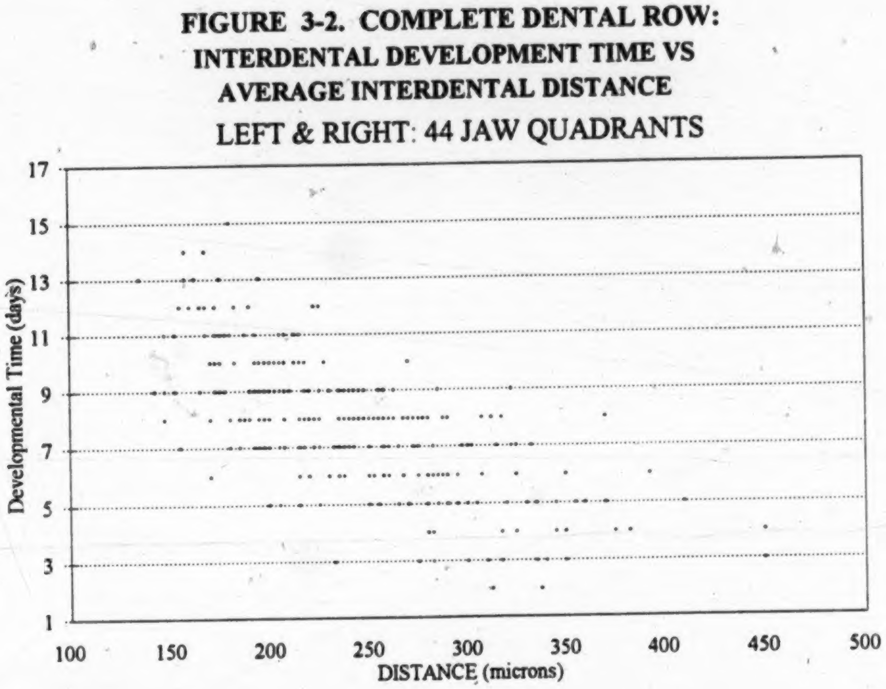


Figure 3-2. The average distance between any pair of adjacent tooth germs in the Complete Dental Row that developed one or more interdental tooth germ(s) relates inversely to the time difference (termed the 'interdental development time') between the establishment of the pair of bordering tooth germs and the identification of the first interdental tooth germ (r: -0.70). All interdental distances and development times were recorded *in vivo* with the dissecting microscope during the recording period (late Stage 54 to Stage 61).

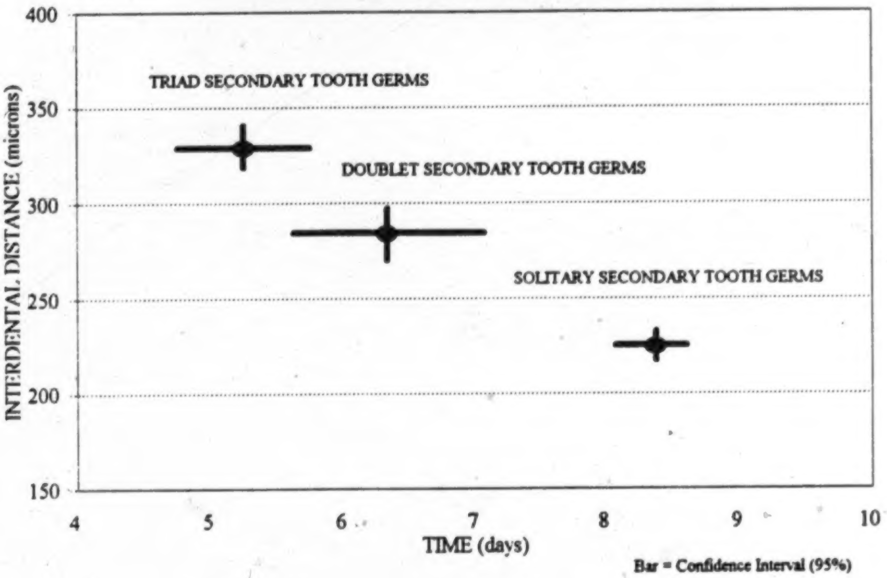


FIGURE 3-3. CDR SECONDARY TOOTH GERMS
AVERAGE INTERDENTAL DISTANCE VS
AVERAGE INTERDENTAL DEVELOPMENT TIME
LEFT & RIGHT: 44 JAW QUADRANTS

Figure 3-3. In the Complete Dental Row (CDR) the average interdental development time for secondary tooth germ types relates to the average distance of the interdental site within which they develop. All interdental distances and development times were recorded *in vivo* with the dissecting microscope during the recording period (late Stage 54 to Stage 61).

FIGURE 3-4. MAXILLARY ORAL EPITHELIUM: AVERAGE THICKNESS VS TIME POSTFERTILIZATION

MID STAGE 54 TO STAGE 59: 24 ANIMALS

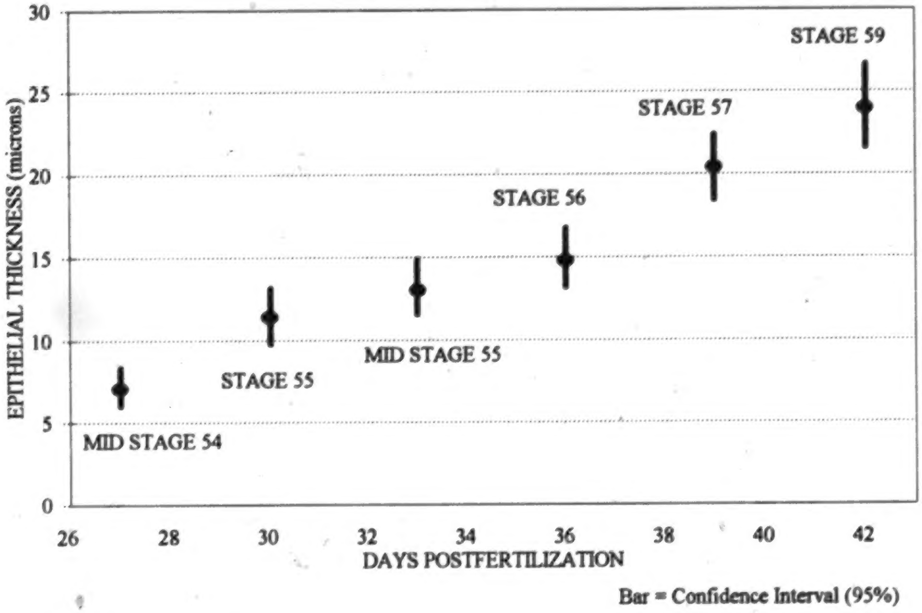


Figure 3-4. The average thickness of the maxillary oral epithelium in the jaw quadrant increased regularly during the Odontogenic Recording Period (ORP). Four animals were selected at six different Stages: mid-54, 55, mid-55, 56, 57 and 59 (Days Post-fertilization: 27, 30, 33, 36, 39, 42, respectively). Each group was processed histologically and the thickness of the epithelium associated with the presumptive and actual dental row was measured. The thickness of the epithelium was adjusted assuming a 30% shrinkage due to histological processing.

FIGURE 3-5. TOOTH GERM INITIATION MODELS

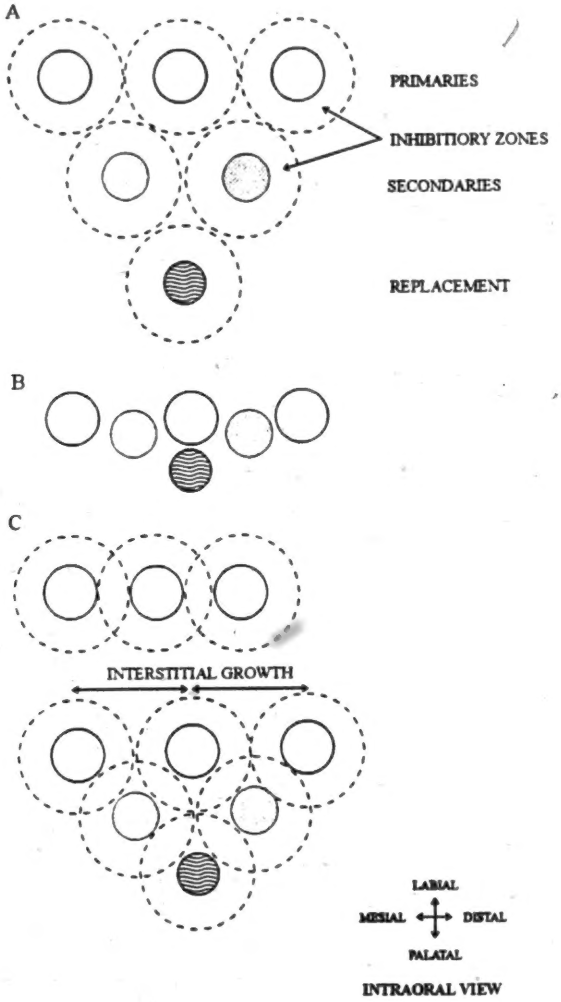

A
PRIMARIES
INHIBITORY ZONES
SECONDARIES
REPLACEMENT
B
C
INTERSTITIAL GROWTH
LABIAL
MESIAL
DISTAL
PALATAL
INTRAORAL VIEW

Figure 3-5. Tooth Germ Initiation Models. Inhibitory zones surrounding developing tooth germs are employed in a number of developmental models to account for the even spacing between teeth. In model **A** the inhibitory zone surrounding the tooth germ does not retract during development, and as a result tooth germs are spaced widely apart. In larval *X. laevis* the tight positioning of the tooth germs suggests that the inhibitory zones associated with primary tooth germs retract prior to the initiation of the secondary and replacement tooth germs (**B**). A popular model for dental inhibitory zones proposes that interstitial grow between the primaries makes space for the interdental teeth (**C**).

FIGURE 3-6. INITIAL DENTAL ROW: DENTAL INITIATION ZONE

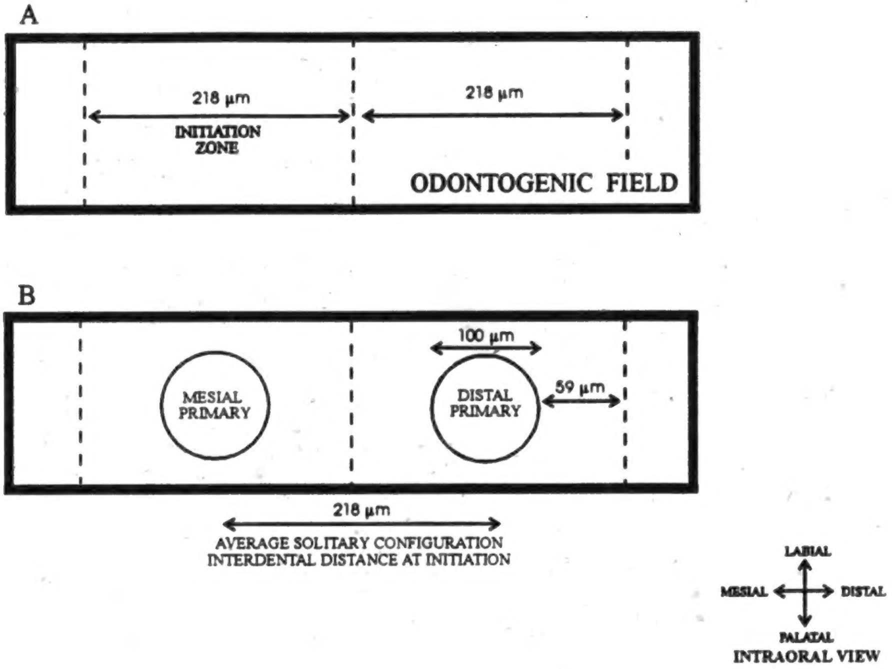

A
218 µm
218 µm
INITIATION ZONE
ODONTOGENIC FIELD
B
100 µm
59 µm
MESIAL PRIMARY
DISTAL PRIMARY
218 µm
AVERAGE SOLITARY CONFIGURATION INTERDENTAL DISTANCE AT INITIATION
LABIAL
MESIAL
DISTAL
PALATAL
INTRAORAL VIEW

Figure 3-6. Dental Initiation Zone in the Initial Dental Row. An initiation zone (IZ) is established once a critical volume of preodontogenic epithlelium is defined in the odontogenic field (OF) during late Stage 54. The width of this zone is speculated to be similar to the interdental distance (218 µm) between primaries which eventually become part of a solitary configuration **(A)**. Dental morphogenesis then proceeds at the center of the IZ with the appearance of a tooth germ **(B)**. Note: For diagramatic purposes the IZs are drawn box-like, but they may well be circular as depicted in the classic models **(Figure 3-5)**.

Figure 3-11. Tooth Germ Initiation in the Triad Dental Configuration. Assuming the 218 μm width of the initiation zone (IZ) in the Initial Dental Row, a 116 μm zone of free preodontogenic tissue exists between the IZs of the primary tooth germs in a triad of average width (**A** and **B**). Retraction of the IZs associated with the primaries then opens a 244 μm zone of free preodontogenic tissue for the establishment of an interdental IZ and the subsequent development of a triad secondary tooth germ (**C** and **D**). Triad tertiary tooth germs appear late in the recording period in a palatal position, gaining the critical epithelial volume for the establishment of their IZ from the palatally extending and thickening preodontogenic epithelium (**E**). Note: For diagramatic purposes the IZs are drawn box-like, but they may well be circular as depicted in the classic models (**Figure 3-9**).

FIGURE 3-7. TOOTH GERM INITIATION: TRIAD DENTAL CONFIGURATION

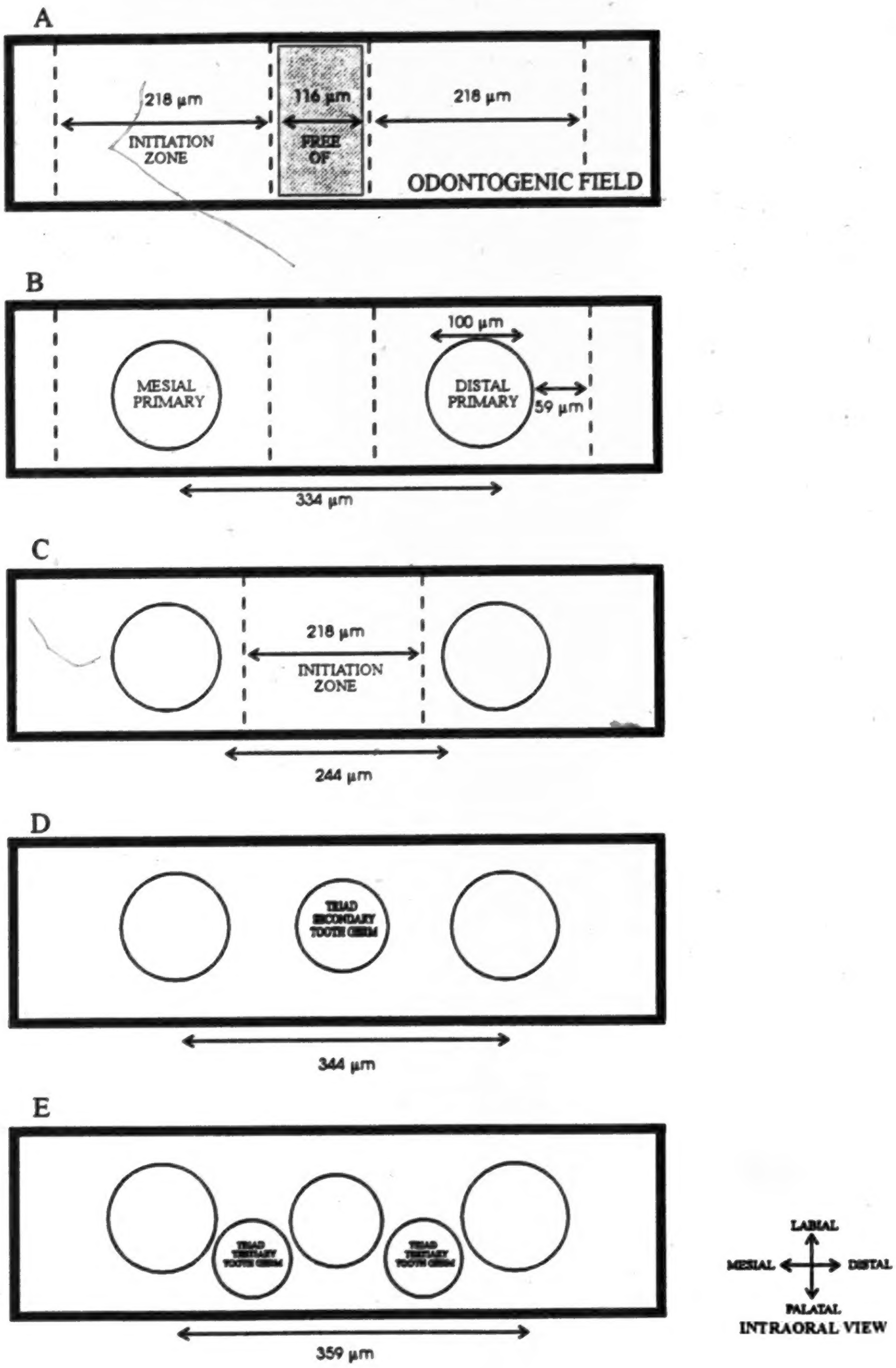

A
218 µm
INITIATION ZONE
116 µm
FREE OF
218 µm
ODONTOGENIC FIELD
B
100 µm
MESIAL PRIMARY
DISTAL PRIMARY
59 µm
334 µm
C
218 µm
INITIATION ZONE
244 µm
D
TRIAD SECONDARY TOOTH GERM
344 µm
E
TRIAD TERTIARY TOOTH GERM
TRIAD TERTIARY TOOTH GERM
359 µm
LABIAL
MESIAL
DISTAL
PALATAL
INTRAORAL VIEW

FIGURE 3-8. TOOTH GERM INITIATION: SOLITARY DENTAL CONFIGURATION

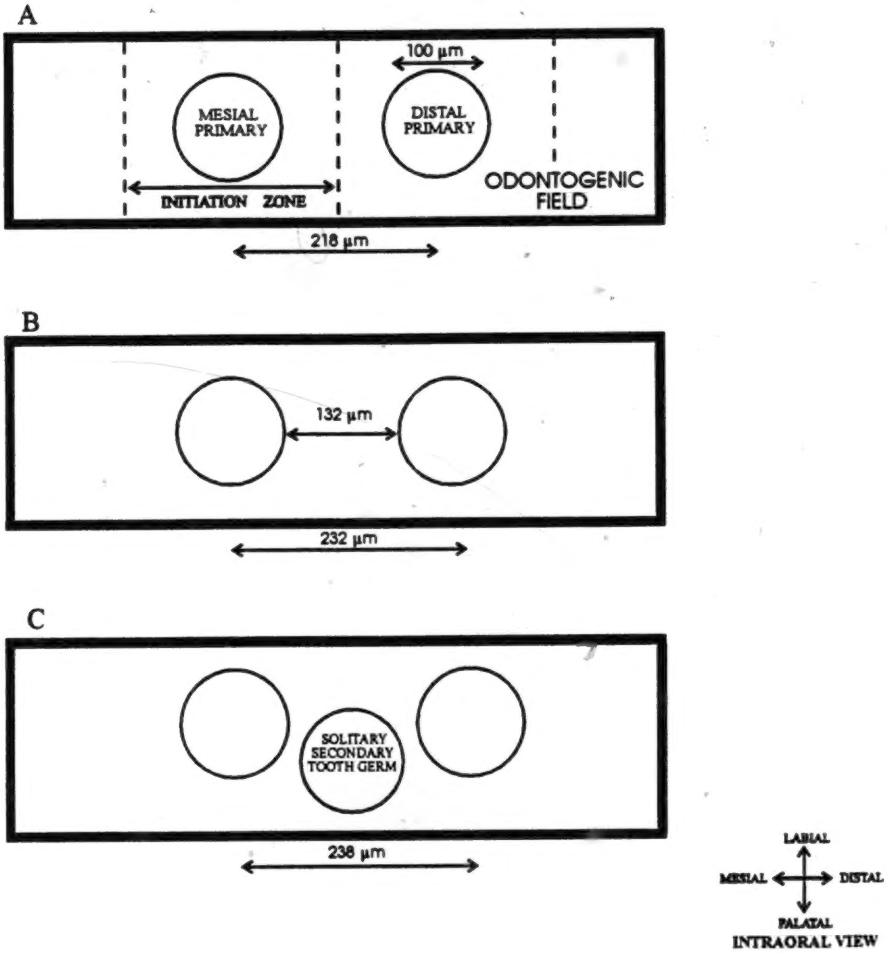

A
100 µm
MESIAL PRIMARY
DISTAL PRIMARY
INITIATION ZONE
ODONTOGENIC FIELD
218 µm
B
132 µm
232 µm
C
SOLITARY SECONDARY TOOTH GERM
238 µm
LABIAL
MESIAL
DISTAL
PALATAL
INTRAORAL VIEW

Figure 3-8. Tooth Germ Initiation in the Solitary Dental Configuration. Assuming the 218 µm width of the initiation zone (IZ) in the Initial Dental Row, only a 132 µm zone of free preodontogenic tissue exists between the margins of the primary tooth germs on the retraction of their IZs (**A** and **B**). As a result, the solitary secondary tooth germ appears later in the recording period in a palatal position, gaining the critical epithelial volume for the establishment of an IZ from the palatally extending and thickening preodontogenic epithelium (**C**). Note: For diagramatic purposes the IZs are drawn box-like, but they may well be circular as depicted in the classic models (**Figure 3-5**).

FIGURE 3-9. TOOTH GERM INITIATION: IDR AND DDR

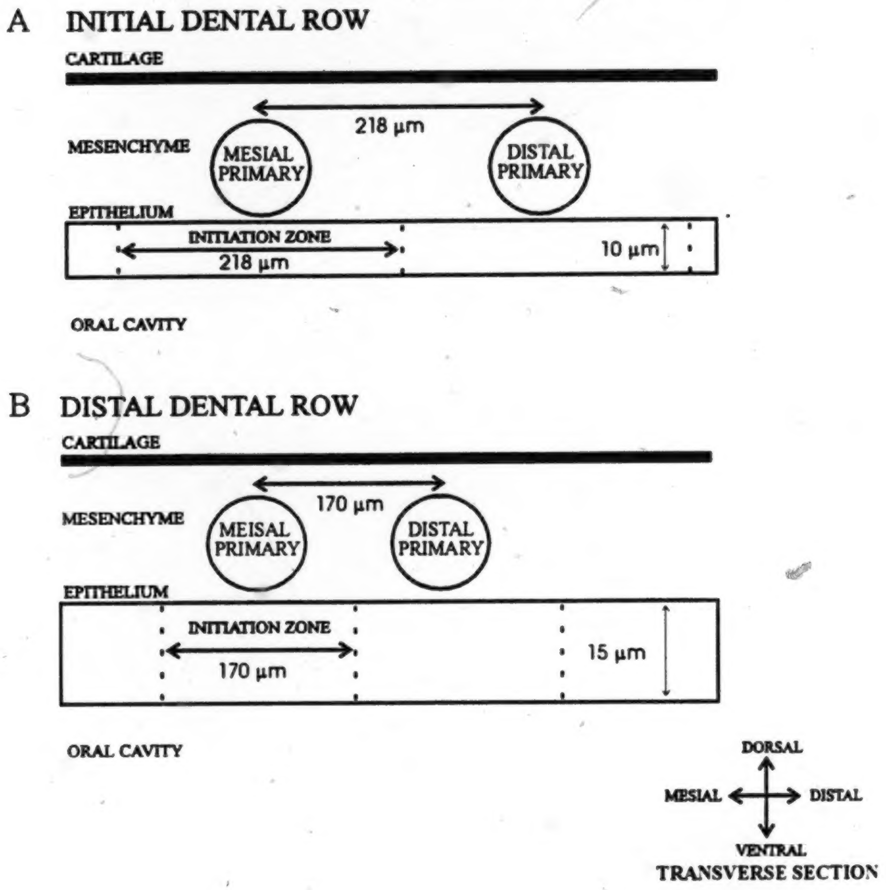
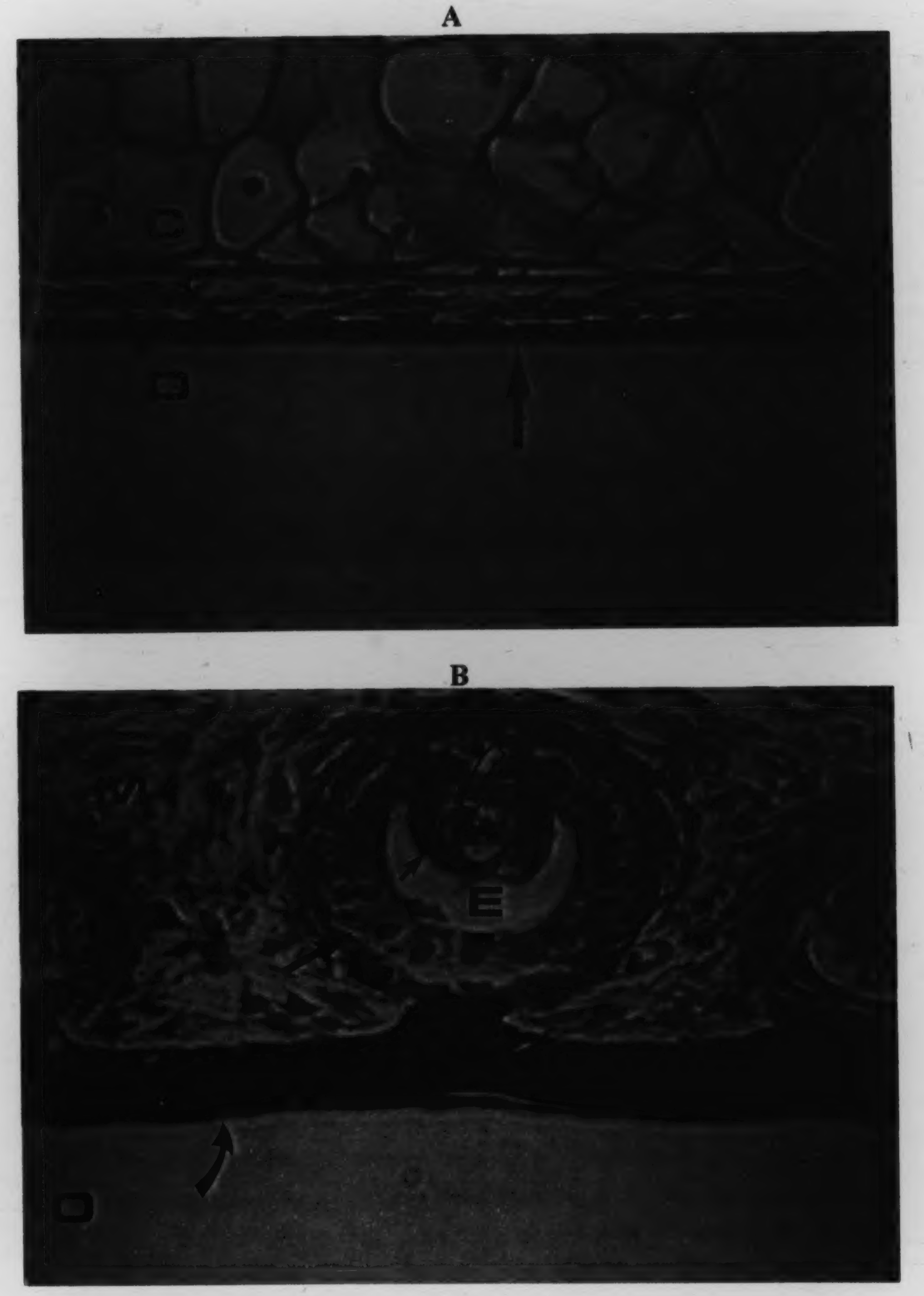

A INITIAL DENTAL ROW
CARTILAGE
218 µm
MESENCHYME
MESIAL PRIMARY
DISTAL PRIMARY
EPITHELIUM
INITIATION ZONE
218 µm
10 µm
ORAL CAVITY
B DISTAL DENTAL ROW
CARTILAGE
170 µm
MESENCHYME
MEISAL PRIMARY
DISTAL PRIMARY
EPITHELIUM
INITIATION ZONE
170 µm
15 µm
ORAL CAVITY
DORSAL
MESIAL
DISTAL
VENTRAL
TRANSVERSE SECTION

Figure 3-9. Tooth Germ Initiation in the Initial Dental Row and Distal Dental Row. The average interdental distance at initiation between primary tooth germs of the solitary configuration was greater in the IDR (218 µm) than the DDR (170 µm). This narrowing in interdental distance could be accounted for by the thickening the preodontogenic epithelium during the recording period. That is, this increasing dimension could account for part of the critical epithelial volume necessary for the establishment of an IZ. The average width of the oral epithelium during the establishment of the IDR was 10 µm (**A**) and it increased to 15 µm midway during the development of the DDR (**B**). Note: The diagram is not drawn to scale.

Plate 3-1A. Transverse section of the upper jaw at mid-Stage 54. Dental development has yet to begin. Arrow points to thin oral epithelium. Suprarostral cartilage (C). Oral mesenchyme (M). Oral cavity (O). Dorsal is toward the top margin of the page. Stained with haematoxylin and eosin. Magnification (X 128).

Plate 3-1B. Transverse section of the upper jaw at Stage 59. Curved closed arrow points to thick oral epithelium. Epithelial dental organ (long closed arrow). Dentine of tooth (short closed arrow). Dental pulp (curved opened arrow). Suprarostral cartilage (C). Oral mesenchyme (M). Oral cavity (O). Enamel space (E). Dorsal is toward the top margin of the page. Stained with haematoxylin and eosin. Magnification (X 128).

PLATE 3-1



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Goin, C.J., and Hester, M. (1961). Studies on the development, succession and replacement of teeth in the frog *Hyla cinerea*. *Journal of Morphology*. **109**, 279-287.

Trueb, L., and Hanken, J. (1992). Skeletal development in *Xenopus laevis* (*Anura: Pipidae*). *Journal of Morphology*. **214, 1-41**.

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Mina, M., Gluhak, J., Upholt, W., Kollar, E.J., and Rogers, B. (1995). Experimental analysis of Msx-1 and Msx-2 gene expression during chick mandibular morphogenesis. *Developmental Dynamics*. **202**, 195-214.

Mina, M., and Kollar, E.J. (1987). The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. *Archives of Oral Biology*. **32**, 123-127.

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CHAPTER 4

TOOTH GERM EXTIRPATION IN THE EMBRYONIC DENTITION: A LONGITUDINAL STUDY IN *XENOPUS LAEVIS* (DAUDIN) BETWEEN STAGES 54 AND 61

A. INTRODUCTION

An important concept in dental embryology asserts that an inhibitory zone surrounds the developing tooth germ. In the dental row these zones are believed to play a significant role in dental initiation and they are a determinative factor in dental patterning. However, this long-held hypothesis has been based on static descriptive studies and has yet to gain experimental support.

Gillette (1955) theorized the existence of dental inhibitory zones in his model of tooth succession in the frog *Rana pipiens*. He proposed a mechanism that consisted of two parts: (1) a tooth germ-initiating potential in the dental lamina that is realized wherever and whenever the conditions of odontogenesis are met, and (2) inhibitory conditions associated with newly initiated tooth germs that prevents the initiation of other tooth germs in the immediate vicinity. Gillette postulated that the inhibitory conditions could be the result of a substance released from the tooth germ or that they were caused by a nutritional debt around the tooth germ which required a recovery period before the initiation of any other teeth. In either case, he concluded that the site in the dental lamina where the inhibitory substance was least concentrated or where the recovery of nutritional resources was first to occur would be midway between two existing tooth germs.

Osborn (1971) described early the development of the dentition in the lizard *Lacerta vivipara* and suggested that a "sphere of inhibition" surrounds the developing tooth. He asserted that it both ensures the even spacing between teeth and regulates the wave replacement of alternate teeth. Osborn (1978, 1984, 1993) later proposed that a discrete group of ectomesenchymal cells (termed a "clone") expands in concert with the growing jaw, giving rise to the dentition. He also speculated that dental initiation is controlled intrinsically and locally within the clone by an inhibitory zone mechanism.

Lumsden (1978, 1979) experimentally investigated Osborn's purported clone but was unable to determine whether it was ectomesenchymal, epithelial or both. He proposed a model that also included an inhibitory zone.

In a study on the embryonic dentition in the alligator *Alligator mississipiensis*, Westergaard and Ferguson (1986, 1987, 1990) also included an inhibitory zone mechanism in their developmental model. They incorporated the current notion that dental patterning resides in the oral epithelium (Mina and Kollar, 1987) and suggested that the initiation of the dentition begins at one locus. This "early initiation stimulus" then spreads through the epithelium and is coupled with an "inhibition process," resulting in zones of competent epithelium capable of inducing the underlying mesenchyme into odontogenesis. This model also has a proliferative aspect as an epithelial "progress zone" develops in concert with jaw growth. Westergaard and Ferguson (1990) noted that new teeth develop at regular distances from older ones and calculated the inhibitory zone to be about 300 μm.

Chapters 2 and 3 offered the first longitudinal description of the development of the early dentition in a study on the tadpole of *Xenopus laevis*. It was suggested that the dentition emerges within an odontogenic field (OF) and that dental initiation begins with the establishment of individual initiation zones (IZ). The tooth germ develops in the center of the IZ which acts as an inhibitory zone not allowing other tooth germ initiations within it. As a result, this mechanism establishes the spacing between tooth germs. Eventually the IZ recedes, opening free zones of preodontogenic tissue and a new IZ is then established between the first tooth germs. Chatper 3 also hypothesized that the width of the IZ varies with the thickness of the dental epithelium. He concluded that dental initiation is intrinsically controlled and proceeds once a critical volume of OF epithelium is established to create an IZ.

Osborn (1971) predicted that the ablation of two adjacent tooth germs should release the inhibitory zone around these teeth and cause the premature initiation of the adjacent teeth. More specifically, a tooth should quickly appear in the interdental space between the ablated teeth, soon followed by the initiation of teeth in the interdental spaces bordering the extirpated teeth. The transparency of *X. laevis* tadpoles and the horizontal orientation of their developing tooth germs makes it possible for the first time both to remove a tooth germ early in development of the dentition and to record longitudinally the impact this procedure has on the developing dental row in the immediate region 2-3 weeks following the surgery.

B. MATERIALS AND METHODS

Animals were obtained by induced breeding with human chorionic gonadotropin (HCG, Sigma Chemical Co., St. Louis, MO). At 4 PM adult females were injected with 800 IU of HCG and placed overnight with adult males which had received 400 IU of HCG at the same time. Embryos developed in gently aerated tap water treated with 1% sodium thiosulphate (2 mL per liter) and maintained at room temperature. Once they were free-swimming tadpoles at 7 days post-fertilization, the animals were transferred to 40 L stock tanks (approximately 200 animals/tank) featuring a one way constant flow (20 L/hr) of filtered (by activated carbon) and dechlorinated (treated with sodium thiosulphate) water held at a constant pH (7.8) and constant temperature (20 ± 0.5 C°). The animals were fed Tadpole Powder (Nasco, Fort Atkinson, WI) sprinkled over the water surface daily at 7 AM and 6 PM. Two varieties of snails aided in maintaining the cleanliness of the tanks. Excess detritus was removed biweekly. A 12 h photo period was maintained (7 AM to 7 PM).

The terminology employed in Chapter 2 to describe the development of the early dentition in larval *X. laevis* is used in this chapter. 'Primary tooth germs' are defined as the first tooth germs to appear in the dental row. Dental development begins quickly over 2 to 4 days during late Stage 54 (Nieuwkoop and Faber, 1967) in the medial two-thirds of the jaw quadrant with a row of 4 to 8 primaries (Initial Dental Row--IDR). These are followed by the distal and sequential addition of more primaries (Distal Dental Row--DDR) over the next 2-3 weeks. On the average, 1 or 2 tooth germs are added to the medial end of the IDR, resulting in the renaming of this row as the 'mesial dental row' (MDR). By Stage 61 the MDR and DDR together form the 'complete dental row' (CDR). In both the MDR and DDR interdental tooth germs develop between the primaries in a more palatal position. A 'secondary tooth germ' develops between two primaries, and together these three tooth germs constitute the 'solitary configuration'. A 'tertiary tooth germ' appears between a secondary and a primary. The arrangement of a secondary and a tertiary between two primaries is a 'doublet'. A pair of tertiaries separated by a secondary and bordered by two primaries results in the 'triad configuration'. The 'null configuration' is a pair of primaries with no interdental tooth between them. [Note: The dental terms 'mesial' and 'distal' are employed in this paper to refer to the 'medial' and 'lateral' aspects of the dental row in each jaw quadrant, respectively (Bitgood and McMahon, 1995; Mina *et al.*, 1995). 'Labial' means 'toward the lip' while 'palatal' indicates 'toward the palate'].

A review of dental development patterns in the IDR from this previous study reveals a relationship between the interdental distance at initiation of the primary tooth germs and the resultant dental configuration (**Figure 4-1**). Triad and doublet incidence increased with the interdental distance. Ninety-six percent of the 148 interdental sites between 125-275 μm developed the solitary configuration. However, a dramatic decrease in the incidence of this configuration to 34.6% occurred at initiation sites that are 300 μm wide, and solitaries failed to appear in sites with an initiation interdental distance above 325 μm.

In the light of this observation, the following criteria were employed in selecting animals for extirpation of a tooth germ:

(1) three primary tooth germs in the IDR that have the central primary usually (but not always) midway between the mesial and distal primaries,

(2) the interdental distance between the centers of the mesial and distal primaries that is not less than 400 μm but not greater than 550 μm,

(3) three primaries that appear on the same day and that are among the first tooth

germs in the mouth, and

(4) three primaries positioned in approximately the middle third of the jaw quadrant.

The following terminology is specific to this chapter. The selected three primary tooth germs are called a 'triplet' (**Plate 4-1A**). The surgical extirpation of the central primary creates a 'surgical triplet' (**Plate 4-1B**). A triplet that has not received the ablative procedure is a 'nonsurgical triplet.' The 'length of a triplet' is defined as the distance between the centers of the mesial and distal primaries. The 'triplet tooth germ number' is the eventual number of tooth germs between the mesial and distal primaries.

Once the tadpoles reached late Stage 54 and the beginning of odontogenesis, animals were selected for the surgical ablation of a tooth germ. They were anesthetized with 2% aminobenzoic acid ethyl ester (MS 222, Sigma Chemical Co., St. Louis, MO), and their mouths were opened with the aid of a retractor fabricated from orthodontic ligature wire. Tooth germ extirpation of the central primary was performed under a binocular dissecting microscope using chemically sharpened tungsten needles. A total of 35 animals received successful surgery on their right side.

The surgical animals were separated into groups of 4 and placed in 10 L brown buckets with the aquatic conditions described above. Small 1 mm incisions in the dorsal epithelium between the olfactory nerves served to identify the tadpoles. A graticule mounted on the microscope was employed to measure the distance between developing tooth germs in the surgical triplets and the dental rows mesial and distal to this site. In order to establish a spatial marker, all measurements were made from the mesial primary of the surgical triplet. Interdental distances were recorded daily until Stage 61 when dramatic metamorphic changes in head morphology and increased tissue opacity made accurate recording impossible. Animals were sacrificed by anesthetic overdose. The dental row was stripped from the underlying suprarostral cartilage (Trueb and Hanken, 1992), in some cases it was stained with methylene blue, and then examined under a culture microscope to establish the final tooth number. Tissue that was processed for histology was fixed in formalin, demineralized with a formic acid and sodium citrate solution, sectioned at 10 μm and stained with haematoxylin and eosin.

Statistical analysis employed the Systat package.

Previous work on the development of the early dentition in *X. laevis* tadpoles (Chapter 3) was employed as a control to establish the developmental patterns for nonsurgical triplets. That study was based on 22 animals which were reared under the identical aquatic conditions of the present investigation.

C. RESULTS

(1) IDR DEVELOPMENT: GENERAL OBSERVATIONS

The development of the dentition in regions of the IDR mesial and distal to the surgical triplet in the 35 experimental animals was examined and compared to the development of the IDR recorded in 22 control animals from the investigation previously cited. Two important relationships emerged.

First, the initiation interdental distance between every pair of primaries that developed a secondary tooth germ varied inversely with the time difference between the establishment of the pair of bordering primaries and the identification of the secondary

(Surgical r: -0.77; Control r: -0.75). Similarly, the average development time required for a secondary to appear at specific initiation interdental distances (150-400 µm) was also comparable between the IDRs in surgical and nonsurgical animals (**Figure 4-7**).

Second, the interdental distance between the primaries at their initiation varied directly with the number of interdental tooth germs that developed (Surgical r: 0.77; Control r: 0.75). A similar relationship emerged in the profile of the dental configuration incidence at specific initiation interdental distances across the IDR for the two animal groups (**Figures 4-1** and **4-2**). In both cases there was a dramatic shift from the solitary configuration to multiple tooth germ configurations at the initiation interdental distances of 275-300 µm.

The average interdental distance between IDR primaries that developed the solitary configuration was similar in both the control animals (218 µm) and the nonsurgical regions of the experimental animals (214 µm). The tooth germ width recorded *in vivo* for both the primaries and secondaries at their initiation was 100 µm in both IDRs.

(2) SURGICAL AND NONSURGICAL TRIPLETS: GENERAL OBSERVATIONS

The average length of a triplet at initiation was similar for both the surgical and control animals (475 µm, range: 400-550 µm, SD: 12; 472 µm, range: 400-550 µm, SD: 13, respectively). The mean final triplet length was slightly longer in the experimental animals (568 µm, range: 450-700 µm, SD: 18; 543 µm, range: 425-650 µm, SD: 13, respectively). As a result, the average growth was only 22 µm greater in the surgical triplets. Moreover, in both contexts the interstitial growth between the primaries at the initiation of their secondaries was similar (surgical triplets: 66 µm, range: 25-125 µm, SD: 10; nonsurgical triplets: 51 µm, range: 25-125 µm, SD: 12).

Two relationships emerged in the surgical triplets that were similar to those in the normally developing dental row. First, the initial length of the surgical triplets related directly with the number of tooth germs that later developed between the mesial and distal primaries (r: 0.72). Second, the interdental development time of the tertiaries in surgical triplets varied inversely (r: -0.78) with the initial interdental distance between their bordering tooth germs.

(3) NONSURGICAL TRIPLETS: TOOTH GERM INITIATION PATTERNS

The nonsurgical triplets had 3 tooth germ initiation pattern types. The most common pattern (53/70) had solitary secondaries develop between the primaries, resulting in 3 tooth germs between the mesial and distal primaries of the triplet (**Figure 4-3A**). That is, this triplet had 2 abutting solitary configurations. The average interdental distance at initiation between the primaries was 231 µm at the mesial site and 230 µm at the distal site. The average times for the secondary to appear at these sites were 8.1 days and 8.3 days, respectively. A less common nonsurgical triplet pattern (12/70) had 1 secondary and 2 tertiaries emerge in one interdental space and a single secondary in the other (**Figure 4-3C**). In other words, this pattern had 5 tooth germs develop between the lateral primaries and featured adjoining triad and solitary configurations. At initiation the average interdental distance of the triad site was 290 µm while it was 231 µm at the solitary site. The average time for the secondary to appear at these sites was 5.8 days and 8.4 days after the establishment of the primaries, respectively. Finally, only 5 of 70 nonsurgical triplets consisted of a doublet and a solitary, resulting in 4 tooth germs between the bordering primary tooth germs (**Figure 4-3B**). **Table 4-1** summarizes the pattern incidence of the nonsurgical triplets, the initial interdental distances between their primaries and the development times of their secondaries.

(4) SURGICAL TRIPLETS: TOOTH GERM INITIATION PATTERNS

The surgical triplets were classified into 7 tooth germ initiation pattern types. The most common (18/35) featured 5 tooth germs between the mesial and distal primaries (**Figure 4-4A**; **Plate 4-2**). More specifically, the '5 tooth germ pattern A' had a pair of secondaries appear soon after the extirpation (average: 4.7 days). The average interdental distance between them was 240 µm. The mean mesial and distal interdental distances were 153 µm and 158 µm, respectively. Toward the end of the ORP, tertiary tooth germs developed in these 3 interdental sites. In nearly three quarters (13/18) of these surgical triplets the central tertiary developed before the mesial and distal tertiaries. The infrequent (2/37) '5 tooth germ B' pattern also had 2 secondary tooth germs emerged quickly (average: 5.5 days), but a pair of tertiaries developed between them (**Figure 4-4B**). The average interdental distance between these secondaries was 325 µm. In both these jaws a mesial tertiary also appeared, but the distal tertiary failed to develop.

Two surgical triplet types had tooth initiation patterns with 4 tooth germs between the mesial and distal primaries. The '4 tooth germ A' pattern (5/35) also had 2 secondaries that quickly appeared following the extirpation procedure (average: 4.9 days; **Figure 4-4C**). However, the average interdental distance between these tooth germs was only 138 μm and there was no central tertiary that developed at this site during the ORP. Mesial and distal tertiaries appeared late in the ORP in interdental sites that averaged 179 μm and 196 μm, respectively. The '4 tooth germ B' pattern (5/35) again had 2 secondaries that developed soon after the surgery (average: 4.6 days). This pattern failed to develop one tertiary tooth germ at either its mesial or distal interdental site during the ORP, though a central tertiary and a tertiary at the other lateral site appeared in every case **(Figure 4-4D)**. The average interdental distance for the non-tooth germ bearing sites was 130 μm.

Three infrequent experimental patterns were noted that developed 3 tooth germs. In contrast to the other surgical triplets, which each had 2 secondaries, the '3 tooth germ A' pattern (3/35) developed only one secondary. It appeared rapidly (average: 4.0 days) at a midpoint between the bordering primaries. Late in the ORP a pair of tertiaries developed on each side of the secondary, offering a pattern identical to the triad configuration **(Figure 4-4E)**. Finally, both '3 tooth germ B' pattern (1/35) and '3 tooth germ C' pattern (1/35) quickly developed 2 secondaries (average: 4.0 days), but only 1 tertiary appeared **(Figures 4-4F and 4-4G)**. In the former, the tertiary developed in the central interdental site while in the latter it appeared in the distal site. The average initiation interdental distance of non-tooth germ bearing sites of both these pattern types was 138 μm. **Table 4-1** summarizes the incidence of the tooth germ initiation patterns in the surgical triplets, the initial interdental distances between their primaries and secondaries, and the development times of their secondaries.

(5) DIFFERENCES BETWEEN SURGICAL AND NONSURGICAL TRIPLET TOOTH GERM INITIATION PATTERNS

The extirpation of the central tooth germ from a triplet dramatically altered the expected tooth germ initiation pattern. Surgical triplets differed from nonsurgical triplets in tooth germ (1) number, (2) type, (3) position, and (4) the development time of the secondaries.

The experimental triplets had on the average one more tooth germ than the nonsurgical triplets (4.4 to 3.4) even though they were only 25 µm longer. More specifically, tooth germ number changed dramatically in the surgical triplets with narrow initial lengths. The majority of nonsurgical triplets (46/53) with initial lengths between 400-500 µm developed 3 tooth germs (**Figure 4-5**). However, in the experimental animals the incidence of 3 tooth germ triplets (5/30) at these initial lengths was sharply reduced and replaced with 4 and 5 tooth germ triplets (**Figure 4-6**). Tooth number significantly increased in the surgical triplets.

In nonsurgical triplets, the tertiaries were associated with the doublet and triad configurations. Since these configurations require a wide initiation interdental distance (greater than 275-300 µm; **Figures 4-1** and **4-2**), they appeared mostly in nonsurgical triplets with initiation interdental distances over 475 µm (14/16; **Figure 4-5**). However, in the surgical triplets the incidence of tertiaries dramatically increased. The average number of tertiaries that develop at specific initiation lengths in both the surgical and nonsurgical triplets increase remarkably in the former. Surgical triplets at all initiation lengths under 500 µm had an average of 2 or more tertiaries. In contrast, only 3 tertiaries were recorded in the 40 nonsurgical triplets with initiation lengths under 500 µm. Tooth type changed in the surgical triplets with a significant increase in tertiary tooth germs.

The majority of surgical triplets (32/35) developed two secondaries, and the mean distance between them at their initiation was 222 µm. This distance was 250 µm in the nonsurgical triplets. Moreover, 29.4% of these in the surgical triplets were under 200 µm compared to 2.8% in the nonsurgical triplets. The average initiation interdental distance of the surgical secondaries from their neighbouring primaries was 160 µm. Twenty-six per cent of these were less than 150 µm. In the nonsurgical triplets, this distance was 125 µm with nearly three times more interdental sites (72.5%) under 150 µm. The position of the secondary tooth germs in surgical triplets differed in that secondaries were closer together, but further from their neighbouring primary.

The first tooth germs to develop following the identification of a triplet were mesial and distal secondaries (excepting the '3 tooth germ A' pattern [3/105]). In nonsurgical triplets, the average development time of these tooth germs regardless their pattern type was 8.0 days. In the surgical triplets with two secondaries, this development time was reduced to 4.7 days. Further, **Figure 4-7** examines secondary tooth development in three regions: (1) the IDR in control animals, (2) the IDR in nonsurgical areas of surgical animals, and (3) the surgical triplet in surgical animals. It plots the average developmental time for each of these classes of secondaries against the initiation interdental distance (150-325 µm) of their primary tooth germs. In the case of surgical secondaries, this distance was the original initiation distance between the mesial and distal primaries and the central primary before extirpation. As previously noted in the nonsurgical sites, the developmental time of the secondaries varied indirectly with the initiation interdental distance of the site in which they developed. However, this relationship failed to appear in the surgical triplets since the average development time for these secondaries at *all* initiation interdental distances was between 4 and 5 days. The interdental sites most dramatically affected were those with the narrowest initiation distances, 150 μm and 175 μm. These had differences in average development time between that expected and that found in the surgical triplets of 7.0 and 6.6 days, respectively. The developmental time of the secondary tooth germs significantly decreased in surgical triplets.

D. DISCUSSION

The surgical extirpation of the central tooth germ in a triplet created tooth germ initiation patterns never before seen in larval *X. laevis*. The resultant patterns were not simply the expected dental configuration less the removed tooth germ. The surgical patterns reflected a new developmental context created after the extirpation, suggesting that dental patterning has an epigenetic or regulative component.

(1) SURGICAL TRIPLET TOOTH GERM PATTERNS: UNIQUE MANIFESTATIONS OF THE DEVELOPMENTAL CONTEXT

The secondary tooth germs that develop in surgical triplets clearly underline an epigenetic aspect in the development of the dentition. They are not merely the manifestation of the expected nonsurgical secondaries. Four lines of evidence support this contention:

1) Surgical secondaries developed on the average 3.3 days sooner than nonsurgical secondaries. This difference was particularly dramatic in narrow initiation interdental sites (150-175 μm) where a secondary was expected to develop in 10-12 days on the average. However, the mean developmental time for all secondaries was between 4 and 5 days. This finding is consistent with Osborn's (1971) prediction that tooth germ extirpation should cause the premature initiation of the neighboring tooth germs.

2) Surgical secondary tooth germs were positioned on the average farther from their bordering primary than nonsurgical secondaries (160 and 125 μm, respectively). This increase in interdental distance of only 35 μm resulted in a significantly greater incidence of tertiary tooth germs at these sites in surgical triplets. In 70 sites, 62 tertiaries appeared (88.5%), while in nonsurgical triplets only 17 tertiaries developed in 140 of these analogous interdental spaces (12.1%). This difference is understandable in light of the fact that the interdental distance of 125 μm appears to be a critical dental initiation width since it is near this distance that the null configuration is found (114 μm; Chapter 3). Increasing this interdental distance by only 35 μm seems to have met the minimum interdental width necessary for the initiation of a tertiary. That is, surgical secondaries were positioned further from their adjacent primaries than expected, and this positional variation provided the critical width for the autonomous initiation of a tertiary. As a result, surgical triplets developed an average of one more tooth than nonsurgical triplets despite being only 25 μm wider at the end of the ORP.

3) In three surgical triplets ('3 tooth germ A' pattern) only one secondary tooth germ

developed instead of the usual two as seen in nonsurgical triplets of the same width. This single tooth germ appeared near the middle of the triplet and was not positioned off to one side near the lateral primary as would be expected if it were a nonsurgical secondary. In these three cases, the resultant dental pattern was identical to the triad configuration.

4) The average interdental distance between the secondary tooth germs was narrower in surgical triplets (222 µm) than in nonsurgical triplets (250 µm) despite the fact that the former triplet was 25 µm wider at the end of the ORP. More significantly, in 29.4% of the surgical triplets these interdental sites were under 200 µm, while only 2.8% of them were below this distance in nonsurgical triplets. The rarity of narrow distances between the secondaries in nonsurgical triplets was expected, since these tooth germs were separated by a primary (100 µm wide). In contrast, secondaries in surgical triplets did not have this structural restriction and they developed at positions never seen in the nonsurgical context. In 5 surgical triplets ('4 tooth germ A' pattern) this interdental space was so narrow (average: 138 µm) that a tertiary never developed during the ORP.

In sum, surgical secondary tooth germs were temporally and positionally distinguishable from the secondaries in the nonsurgical triplets. This suggests that at the time the primaries of the IDR appeared under the dissecting microscope the secondaries had yet to be established at any biological level. More specifically, the initiation of secondaries was epigenetically determined by (i.e., regulated by) the immediate developmental context of the preodontogenic tissue created after the extirpation of the central tooth germ.

(2) SURGICAL TRIPLET TOOTH GERM INITIATION PATTERNS: CHARACTERISTIC MANIFESTATIONS OF A DEVELOPMENTAL PROGRAM

Dental development in the surgical triplets, though distinctive, was also similar to that in the nonsurgical dental row. Four lines of evidence support this contention:

1) The number of tooth germs that developed varied directly with the initial length of a surgical triplet (r: 0.72). This distance to tooth number relationship also existed in the IDR between primary tooth germs in both the control animals (r: 0.75) and the nonsurgical regions of the experimental animals (r: 0.77).

2) The development time of the tertiary tooth germs related inversely with the width of the interdental sites in the surgical triplets (r: -0.78). This interdental distance to development time relationship was also found in the IDR between primaries in both the control animals (r: -0.75) and the experimental animals (r: -0.77).

3) The surgical triplets featured 7 recognizable dental developmental patterns, suggesting a random element in dental initiation similar to that seen in the IDR. The positions of the secondary tooth germs in surgical triplets ultimately shaped the final dental pattern in a manner similar to that effected by the primaries in normal development. For example, secondaries positioned close together (average: 138 μm) or near to the bordering primary (average: 125 μm) failed to develop a tertiary at the narrow site. In other words, dental initiation was intrinsically controlled at the initiation sites, including surgical triplets, and was not controlled by an overarching mechanism (e.g., the Zahnreihen Theory).

4) In the surgical triplets, odontogenic potential migrated palatally during the ORP

as the secondaries and more so the tertiaries were positioned progressively more palatally. These spatial and temporal characteristics of the tooth germs were also seen in normal development (**Figure 4-3** and **4-4**).

In sum, the surgical triplets, despite manifesting tooth germ initiation patterns distinct from the normal dental row, shared with the latter a basic dental developmental process.

(3) SURGICAL TRIPLET TOOTH GERM INITIATION PATTERNS: AN ODONTOGENIC FIELD THEORY INTERPRETATION

Chapter 3 proposed the Odontogenic Field Theory based on a descriptive study of early dental development in larval *X. laevis*. This theory suggests the existence in the jaw of an epithelial odontogenic field (OF) within which individual initiation zones (IZ) compete to establish themselves. Once a critical mass of epithelial cells is established, an IZ is created and a tooth germ then develops near its center. The IZ temporarily acts as an inhibition zone that restricts the development of any other tooth germs within it. This restrictive zone later recedes freeing up preodontogenic tissue bordering the newly formed tooth germ. Once the necessary conditions for dental initiation in this adjacent preodontogenic tissue are met, a new IZ is established and another tooth germ develops. In Chapter 3, it was speculated that dental patterning reflects the competition between the IZs and concluded that dental initiation is autonomous, being intrinsically and locally controlled at the sites of initiation. Moreover, dental initiation, and ultimately dental patterning, in *X. laevis* tadpoles has an epigenetic component.

In Chapter 3, it was argued that the width of the IZ was reflected by the average initiation interdental distance between IDR primaries which developed into the solitary configuration (217 µm). That is, this configuration represented the edge-to-edge butting of IZ margins at the time of their establishment (Chapter 3, **Figure 3-6**). In the present investigation, this distance in the nonsurgical regions of the experimental animals was 214 µm. The mean width of primaries at initiation was 100 µm; thus at this time each tooth germ was bordered on either side by a 57 µm margin of IZ tissue.

Figure 4-8 schematizes the developmental dynamic and patterning of the average surgical triplet in the light of the Odontogenic Field Theory. The simultaneous development of three IDR primary tooth germs suggests that their IZs were established at about the same time. However, the 237 µm average distance between these primaries reflects the triplet selection criteria and suggests that narrow gaps of free OF tissue (237 µm - 214 µm = 23 µm) separated their IZs at initiation (**Figures 4-8A** and **8B**). At the time the secondaries appeared, the mean distance between the lateral margins of the neighbouring primaries was 442 µm, a distance which could accommodate two IZs of the standard 214 µm width (**Figure 4-8C**). The coincidental appearance of a pair of secondaries suggests that their IZs were established at the same time. More specifically, the average interdental distance at initiation between the secondaries (222 µm) argues that the borders of these IZs nearly butted up against each other. In contrast, the average initiation interdental distance between the surgical secondaries and bordering primaries (160 µm) indicated the loss of the inhibitory effect from the IZ of the primaries, a situation similar to the initiation of a triad secondary tooth germ in normal development (Chapter 3, **Figure 3-7C**). As a result, in both the triad and surgical triplet, the secondary was positioned near the primary (average: 167 µm and 160 µm, respectively). The margin of IZ tissue associated with these tooth germs at the central interdental site (61 µm) and that between these secondaries and their lateral primaries (mesial site: 54 µm distal site: 65 µm) is consistent with the standard 57 µm margin of IZ tissue of newly initiated tooth germs in the nonsurgical regions of the IDR development (**Figure 4-8D**). Finally, the critical epithelial mass for tertiary tooth germ development is made available through the recession of the secondaries' IZs, the extension of the OF palatally and the thickening of the OF epithelium (**Figure 4-8E**).

To conclude, surgical triplets had characteristics that were both similar to and dissimilar from normal dental row development. The similarities reflect a basic dental development program which is manifested within a developmental context. The surgical extirpation of the central tooth germ in a triplet modified the developmental context in which the basic dental program is expressed, resulting in dental developmental patterns not seen in normal development.

Table 4-1. The average interdental distance between tooth germs at initiation for different sites in both the surgical and nonsurgical triplets is presented along with the average development times of those sites which develop secondary tooth germs. All interdental distances and development times were recorded *in vivo* with the dissecting microscope during the recording period (late Stage 54 to Stage 61). See **Figures 4-3** and **4-4** to identify the interdental sites and their respective secondary tooth germs. The tooth germ initiation patterns in surgical triplets (5 Tooth Germ [B], 4 Tooth Germ [B] and 3 Tooth Germ [C]) feature a non-tooth bearing interdental site that may be positioned adjacent either the mesial or distal primary tooth germ. The interdental distance recorded in the table reflects whether the site was 'Tooth Bearing' or had 'Non-Tooth Bearing,' and does not indicate the position of the interdental site in the surgical triplet.

TABLE 4-1. NONSURGICAL AND SURGICAL TRIPLETS: AVERAGE INTERDENTAL DISTANCE AT INITIATION AND AVERAGE DEVELOPMENT TIME OF SECONDARY TOOTH GERMS

	N	INTERDENTAL DISTANCE Average (St Dev) Range (microns)	DEVELOPMENT TIME Average (St Dev) Range (days)	INTERDENTAL DISTANCE Average (St Dev) Range (microns)	DEVELOPMENT TIME Average (St Dev) Range (days)	INTERDENTAL DISTANCE Average (St Dev) Range (microns)	DEVELOPMENT TIME Average (St Dev) Range (days)
NONSURGICAL TRIPLETS	/70						
A. 3 TOOTH GERM	53						
CONFIGURATION		SOLITARY	SOLITARY	SOLITARY	SOLITARY		
		MESIAL SITE	2ND TG	DISTAL SITE	2ND TG		
		231 (9)	8.1 (1.7)	230 (9)	8.3 (1.9)		
		150-300	3-13	175-300	3-13		
B. 4 TOOTH GERM	5						
CONFIGURATION		SOLITARY	SOLITARY	DOUBLET	DOUBLET		
		SITE	2ND TG	SITE	2ND TG		
		228 (54)	8.8 (3.0)	256 (38)	7.5 (1.0)		
		175-250	7-11	225-300	5-8		
C. 5 TOOTH GERM	12						
CONFIGURATION		SOLITARY	SOLITARY	TRIAD	TRIAD		
		SITE	2ND TG	SITE	2ND TG		
		231 (22)	8.4 (2.2)	290 (20)	5.8 (1.8)		
		200-250	6-13	250-300	3-8		
SURGICAL TRIPLETS	/35						
A. 5 TOOTH GERM (A)	18	MESIAL	MESIAL	CENTRAL		DISTAL	DISTAL
		SITE	2ND TG	SITE	2ND TG	SITE	2ND TG
		153 (8)	4.6 (1.1)	240 (12)	N/A	158 (5)	4.8 (0.9)
		100-250	3-7	150-350		125-200	3-6
B. 5 TOOTH GERM (B)	2	TOOTH BEARING	MESIAL	CENTRAL		NON-TOOTH	DISTAL
		SITE	2ND TG	SITE	2ND TG	BEARING SITE	2ND TG
		150 (-)	4 (-)	325 (-)	N/A	150 (-)	7 (-)
C. 4 TOOTH GERM (A)	5	MESIAL	MESIAL	CENTRAL		DISTAL	DISTAL
		SITE	2ND TG	SITE	2ND TG	SITE	2ND TG
		179 (25)	4.5 (0.6)	138 (21)	N/A	196 (25)	5.3 (0.8)
		160-200	4-5	100-150		175-225	5-7
D. 4 TOOTH GERM (B)	5	NON-TOOTH	MESIAL	CENTRAL		TOOTH BEARING	DISTAL
		BEARING SITE	2ND TG	SITE	2ND TG	SITE	2ND TG
		125 (0.0)	4.6 (1.4)	225 (30)	N/A	185 (28)	4.6 (0.6)
		125	4-5	200-275		150-225	3-6
E. 3 TOOTH GERM (A)	3	MESIAL	MESIAL	CENTRAL		DISTAL	DISTAL
		SITE	2ND TG	SITE	2ND TG	SITE	2ND TG
		225 (0.0)	NA	NA	4 (1.0)	225 (1.0)	NA
		225	NA	NA	3-5	8-10	NA
F. 3 TOOTH GERM (B)	1	MESIAL	MESIAL	CENTRAL		DISTAL	DISTAL
		SITE	2ND TG	SITE	2ND TG	SITE	2ND TG
		150 (-)	3 (-)	250 (-)	N/A	100 (-)	5 (-)
		NA	NA	NA	NA	NA	NA
G. 3 TOOTH GERM (C)	1	TOOTH BEARING	MESIAL	CENTRAL		NON-TOOTH	DISTAL
		SITE	2ND TG	SITE	2ND TG	BEARING SITE	2ND TG
		175 (-)	4 (-)	150 (-)	N/A	125 (-)	4 (-)
		NA	NA	NA	NA	NA	NA

**FIGURE 4-1. INITIAL DENTAL ROW:
DEVELOPMENTAL TOOTH GERM CONFIGURATION VS
INITIATION INTERDENTAL DISTANCE**
22 CONTROL ANIMALS: 204 SITES

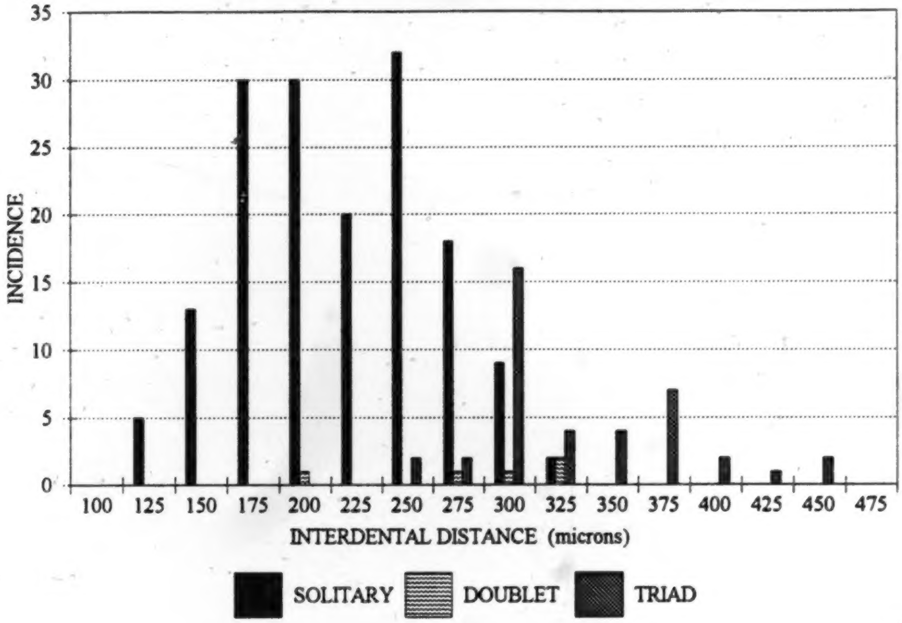


Figure 4-1. In the Initial Dental Row (IDR) of control animals, the profile of developmental tooth germ configuration (solitary, doublet, triad) incidence reflects the interdental distance between the primary tooth germs at their initiation. The final tooth germ number was determined histologically with the culture microscope at Stage 61 after the dental row was stripped from the suprarostral cartilage.

FIGURE 4-2. INITIAL DENTAL ROW:
DEVELOPMENTAL TOOTH GERM CONFIGURATION VS
INITIATION INTERDENTAL DISTANCE
35 SURGICAL ANIMALS: 88 NONSURGICAL SITES

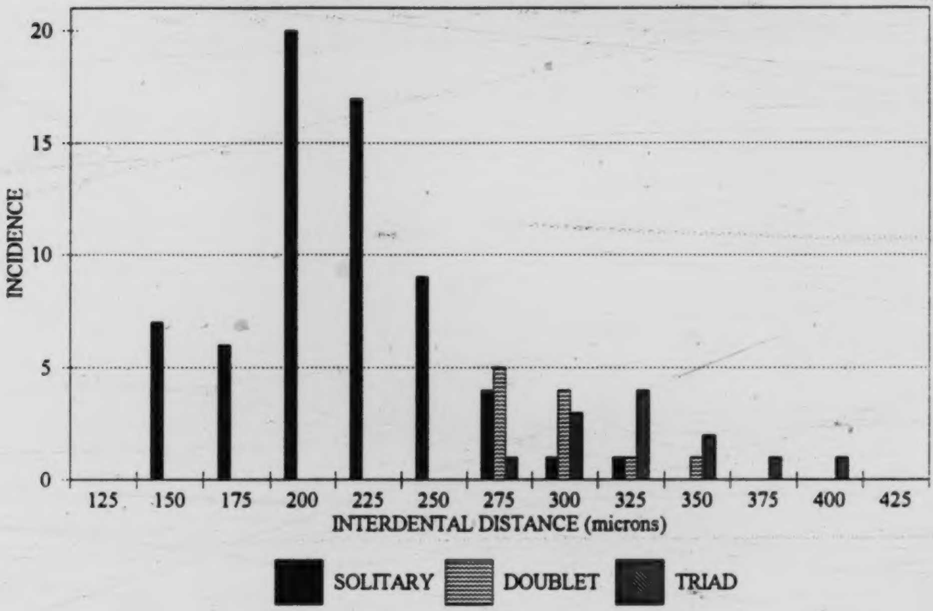


Figure 4-2. In the Initial Dental Row (IDR) of surgical animals on each side of a surgical triplet, the profile of developmental tooth germ configuration (solitary, doublet, triad) incidence reflects the interdental distance between the primary tooth germs at their initiation. The final tooth germ number was determined histologically with the culture microscope at Stage 61 after the dental row was stripped from the suprarostral cartilage.

FIGURE 4-3. NONSURGICAL TRIPLETS:
TOOTH GERM INITIATION PATTERNS

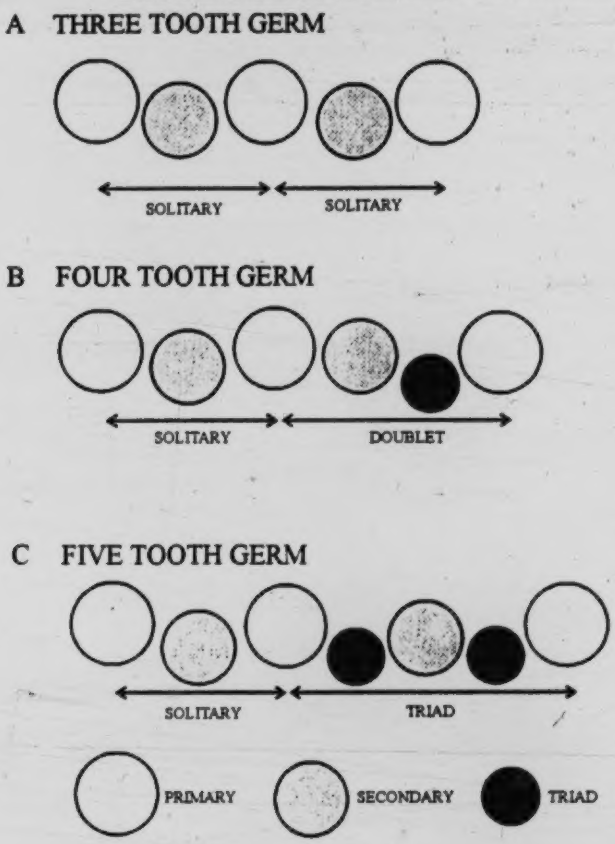
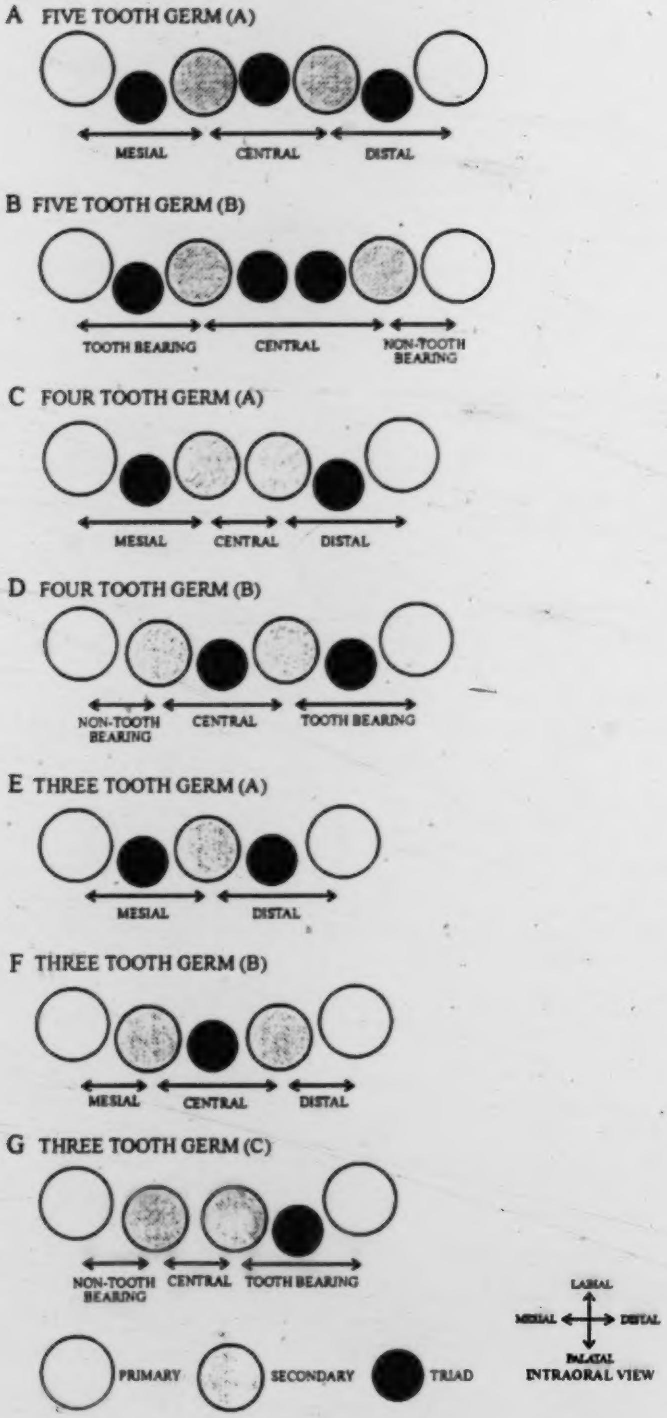

A THREE TOOTH GERM
SOLITARY
SOLITARY
B FOUR TOOTH GERM
SOLITARY
DOUBLET
C FIVE TOOTH GERM
SOLITARY
TRIAD
PRIMARY
SECONDARY
TRIAD

Figure 4-3. Tooth Germ Initiation Patterns in Nonsurgical Triplets. Three pattern types (**A-C**) were noted in the Initial Dental Row (IDR) of control animals. The incidence of these are presented in **Table 4-1**. The final tooth germ number was determined histologically with the culture microscope at Stage 61 after the dental row was stripped from the suprarostral cartilage.

Figure 4-4. Tooth Germ Initiation Patterns in Surgical Triplets. Seven pattern types (A-G) were noted in the surgical triplets of experimental animals. The initiation patterns '5 Tooth Germ (B),' '4 Tooth Germ (B)' and '3 Tooth Germ (C)' feature a non-tooth bearing interdental site that could be positioned adjacent either the mesial or distal primary tooth germ. For diagramatic purposes, these mesial and distal interdental sites are demarcated either 'Tooth Bearing' or 'Non-Tooth Bearing.' The incidence of the surgical tooth germ initiation patterns is presented in **Table 4-2**. The final tooth germ number was determined histologically with the culture microscope at Stage 61 after the dental row was stripped from the suprarostral cartilage.

FIGURE 4-4. SURGICAL TRIPLETS:
TOOTH GERM INITIATION PATTERNS



A FIVE TOOTH GERM (A)

B FIVE TOOTH GERM (B)

C FOUR TOOTH GERM (A)

D FOUR TOOTH GERM (B)

E THREE TOOTH GERM (A)

F THREE TOOTH GERM (B)

G THREE TOOTH GERM (C)

MESIAL
CENTRAL
DISTAL
TOOTH BEARING
CENTRAL
NON-TOOTH BEARING
MESIAL
CENTRAL
DISTAL
NON-TOOTH BEARING
CENTRAL
TOOTH BEARING
MESIAL
DISTAL
MESIAL
CENTRAL
DISTAL
NON-TOOTH BEARING
CENTRAL
TOOTH BEARING
LABIAL
MESIAL
DISTAL
PALATAL
INTRAORAL VIEW
PRIMARY
SECONDARY
TRIAD

FIGURE 4-5. NONSURGICAL TRIPLETS:
TOOTH NUMBER INCIDENCE VS TRIPLET INITIAL LENGTH
22 CONTROL ANIMALS: 70 NONSURGICAL TRIPLETS

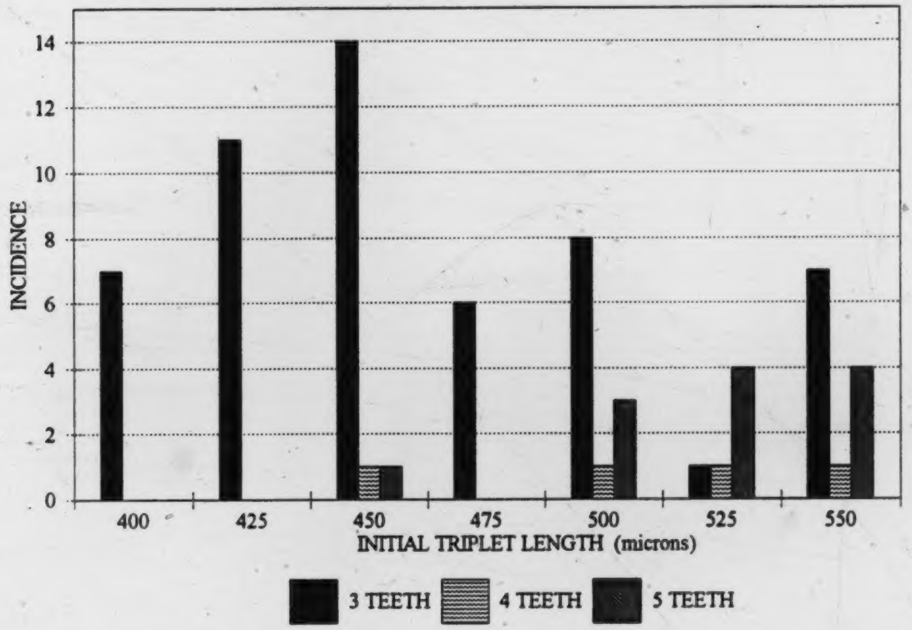


Figure 4-5. The incidence of the number of tooth germs that develop during the recording period in a nonsurgical triplet of a control animal is profiled against the length of the triplet at initiation. The final tooth germ number does not include the mesial and distal primaries and it was determined histologically with the culture microscope at Stage 61 after the dental row was stripped from the suprarostral cartilage.

FIGURE 4-6. SURGICAL TRIPLETS:
TOOTH NUMBER INCIDENCE VS TRIPLET INITIAL LENGTH
35 ANIMALS: 35 SURGICAL TRIPLETS

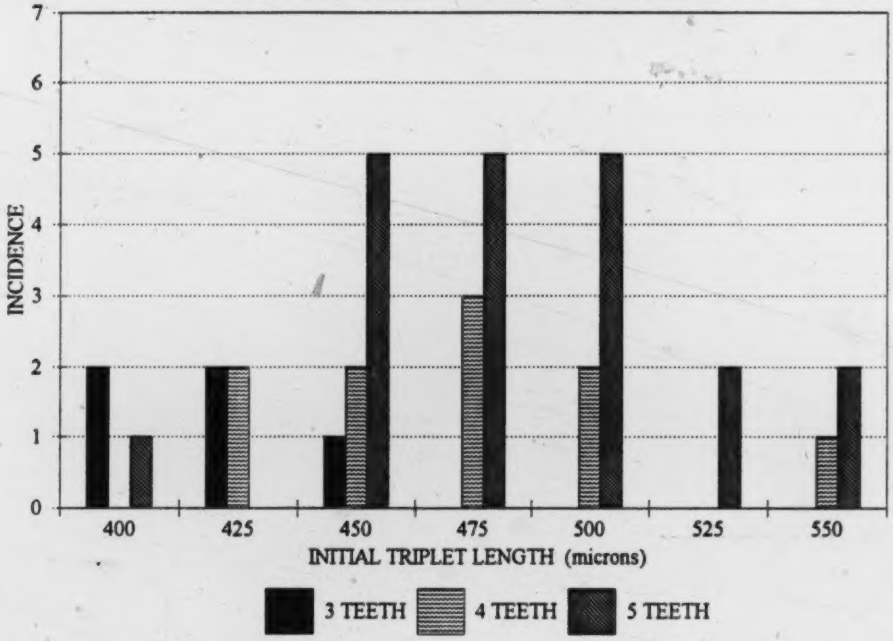


Figure 4-6. The incidence of the number of tooth germs that develop during the recording period in a surgical triplet of an experimental animal is profiled against the length of the triplet at initiation. The final tooth germ number does not include the mesial and distal primaries and it was determined histologically with the culture microscope at Stage 61 after the dental row was stripped from the suprarostral cartilage.

FIGURE 4-7. TRIPLET SECONDARY TOOTH GERMS: AVERAGE INTERDENTAL DEVELOPMENT TIME VS INITIATION INTERDENTAL DISTANCE

35 SURGICAL ANIMALS: 88 NONSURGICAL & 67 SURGICAL SITES
22 CONTROL ANIMALS: 204 INTERDENTAL SITES

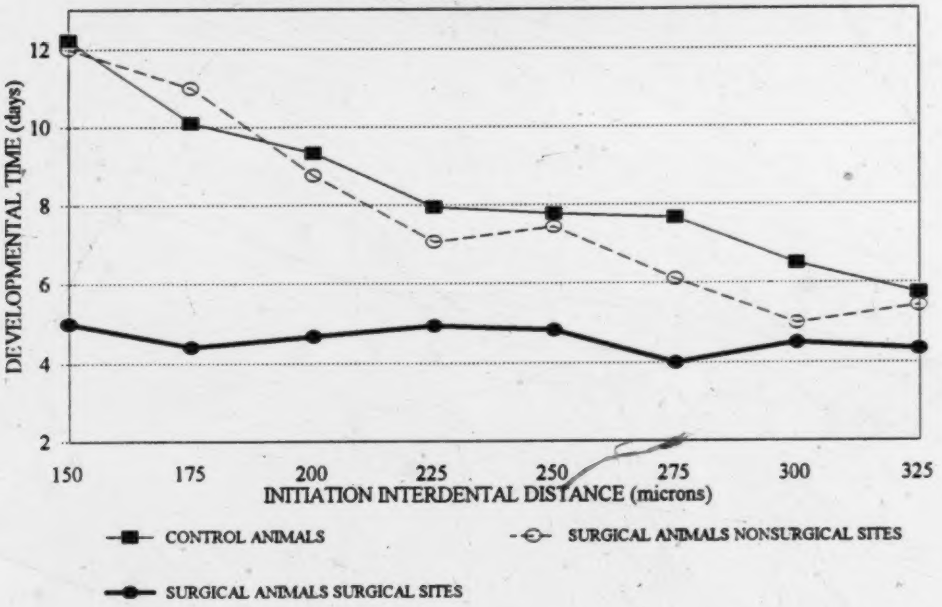


Figure 4-7. The average interdental development time for secondary tooth germs at specific initiation interdental distances of their bordering primary tooth germs is compared in three developmental contexts: (1) the IDR in control animals, (2) the IDR in nonsurgical regions of surgical animals, and (3) the surgical triplet in surgical animals. All interdental distances and development times were recorded *in vivo* with the dissecting microscope during the recording period (late Stage 54 to Stage 61).

Figure 4-8. Tooth Germ Initiation in the Surgical Triplet. Assuming the 214 μm width of the initiation zone (IZ) based on the nonsurgical regions of the Initial Dental Row in experimental animals, a 23 μm zone of free preodontogenic tissue exists between the IZs of the primary tooth germs in a surgical triplet of average width (**A** and **B**). With the extirpation of the central primary tooth germ of the triplet and the retraction of the IZs associated with the mesial and distal primaries, a 442 μm zone of free preodontogenic tissue is opened for the establishment of two IZs and the subsequent development of two surgical secondary tooth germs (**C** and **D**). Surgical tertiary tooth germs appear late in the recording period in a palatal position, gaining the critical epithelial volume for the establishment of an IZ from the palatally extending and thickening preodontogenic epithelium (**E**). The tooth germ initiation depicted if the Five Tooth Germ (A) pattern which appeared in half of the surgical triplets.

FIGURE 4-8. TOOTH GERM INITIATION: SURGICAL TRIPLET

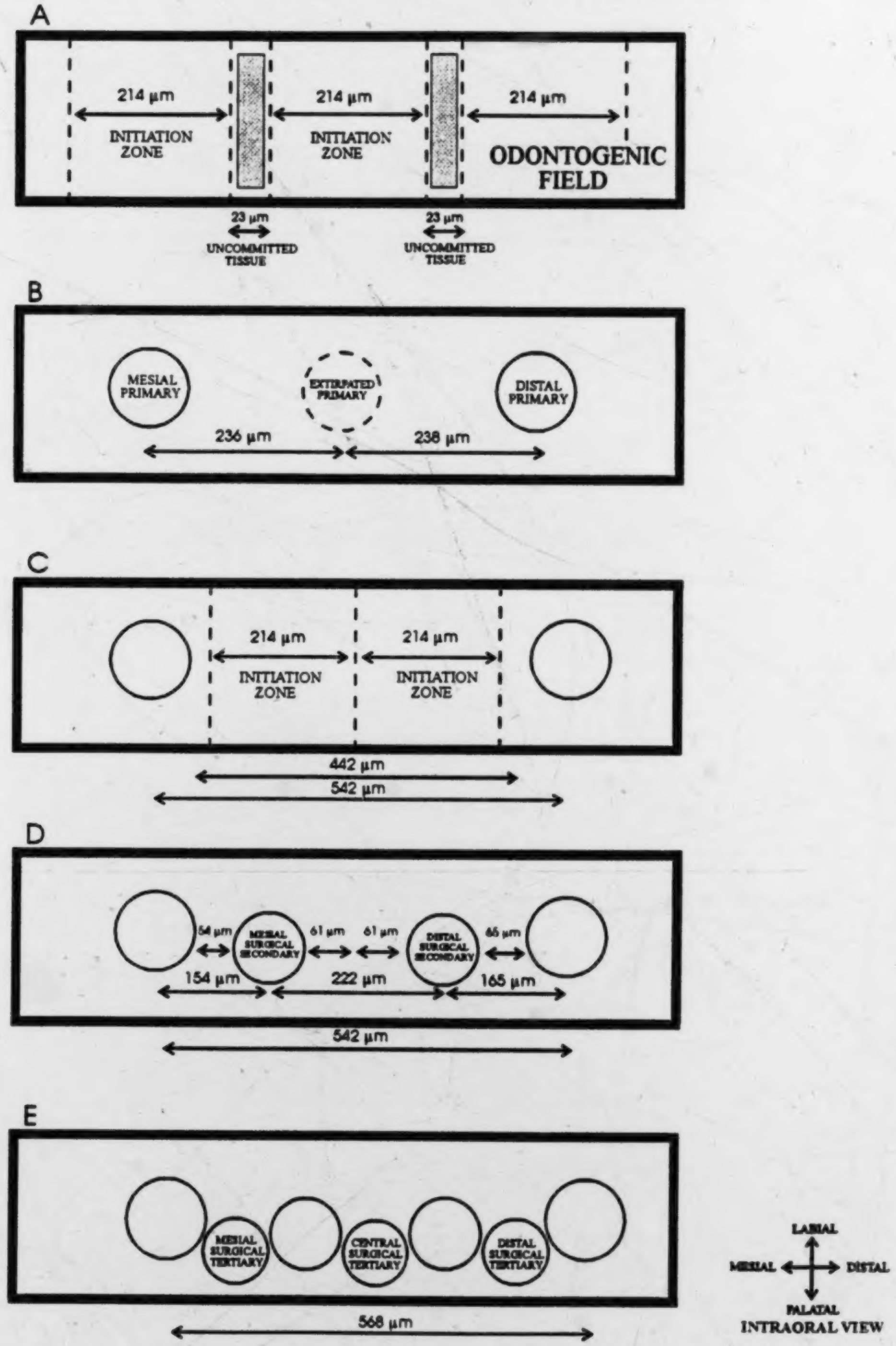
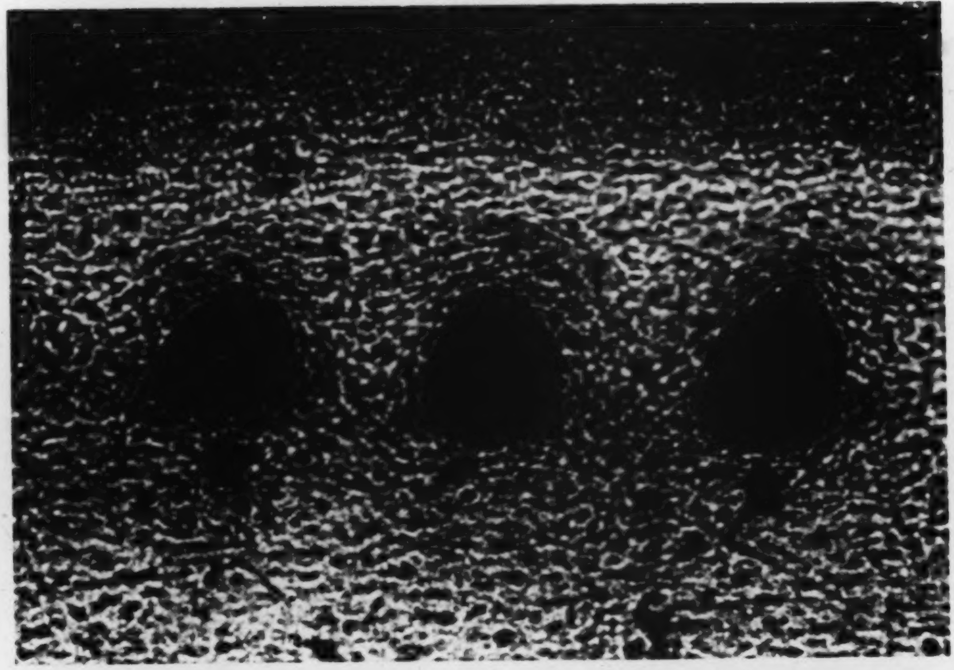

A
214 µm
INITIATION ZONE
214 µm
INITIATION ZONE
214 µm
ODONTOGENIC FIELD
23 µm
UNCOMMITTED TISSUE
23 µm
UNCOMMITTED TISSUE
B
MESIAL PRIMARY
EXTIRPATED PRIMARY
DISTAL PRIMARY
236 µm
238 µm
C
214 µm
INITIATION ZONE
214 µm
INITIATION ZONE
442 µm
542 µm
D
54 µm
MESIAL SURGICAL SECONDARY
61 µm
61 µm
DISTAL SURGICAL SECONDARY
66 µm
154 µm
222 µm
165 µm
542 µm
E
MESIAL SURGICAL TERTIARY
CENTRAL SURGICAL TERTIARY
DISTAL SURGICAL TERTIARY
568 µm
LABIAL
MESIAL
DISTAL
PALATAL
INTRAORAL VIEW

Plate 4-1A. Dental triplet at late Stage 54. Mesial primary tooth germ (left arrow), central primary and distal primary (right arrow). The dental row was stripped from underlying suprarostral cartilage and viewed under the culture microscope. Labial is toward the top margin of the page. Stained with methylene blue. Magnification (X 51).

Plate 4-1B. Surgical triplet at late Stage 54 immediately following the extirpation of the central primary tooth germ. Extirpation site (E). Mesial (left arrow) and distal (right arrow) primaries. The dental row was stripped from underlying suprarostral cartilage and viewed under the culture microscope. Labial is toward the top margin of the page. Stained with methylene blue. Magnification (X 51).

PLATE 4-1

A



B

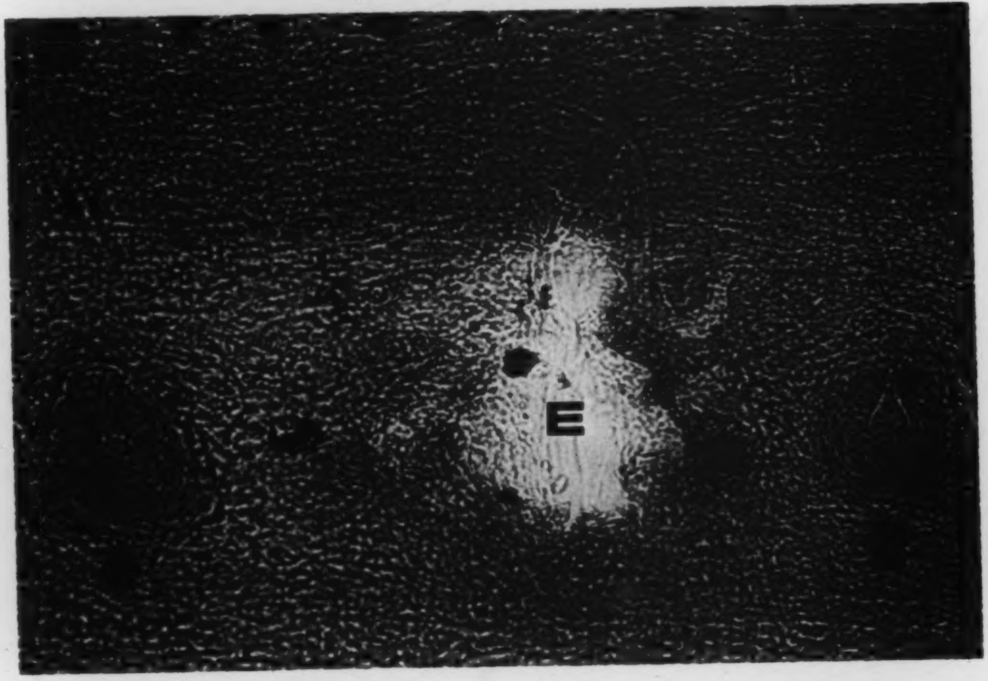


PLATE 2-5

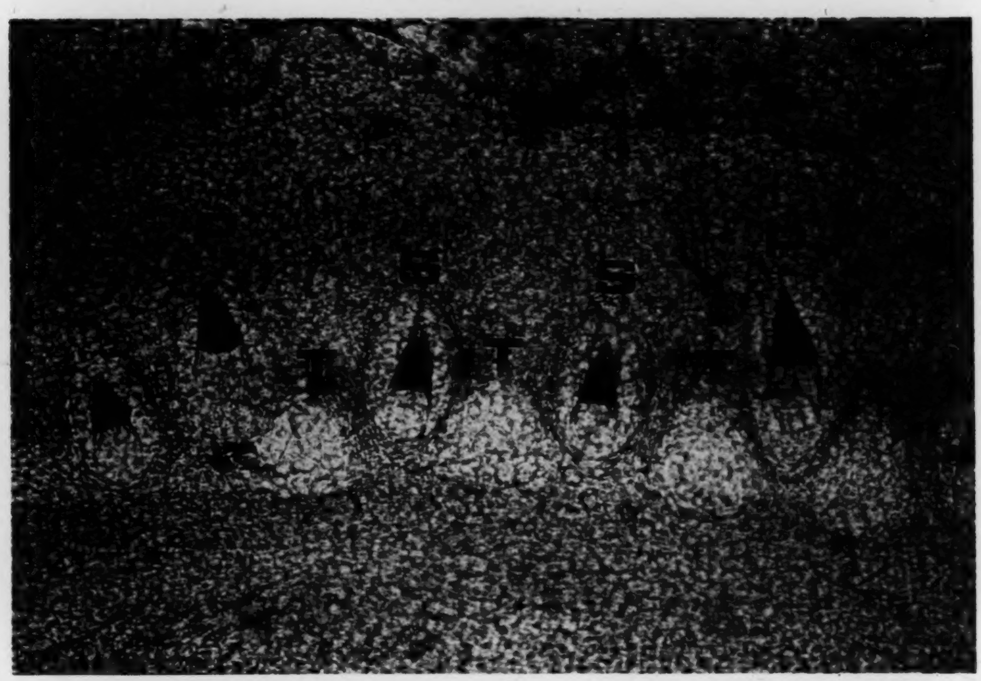


Plate 4-2. Five tooth germ pattern (A) surgical triplet. Primaries (P). Secondaries (S). Tertiaries (T). The dental row was stripped from the suprarostral cartilage and viewed under culture microscope. Labial is toward the top margin of the page. Unstained. Magnification (X 32).

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CHAPTER 5

EXTIRPATION EXPERIMENTS IN THE EMBRYONIC DENTITION: A LONGITUDINAL STUDY IN XENOPUS LAEVIS (DAUDIN) BETWEEN STAGES 40 AND 61

A. INTRODUCTION

Developing teeth have provided a fruitful model for the investigation of basic embryological mechanisms. Knowledge of individual tooth development is extensive, yet not much is known of the mechanism that establishes the overall pattern of the dentition. More specifically, theories of the development of the dentition have been based on static descriptive studies and have little experimental support. Despite the limited understanding of this subject, four developmental models continue to be discussed in the literature.

Butler (1939, 1956, 1978, 1995) suggested that different embryological dental fields account for mammalian heterodonty. The Dental Field Theory proposed that a series of identical dental primordia are initiated across the jaw and that they develop in accordance with the dental field in which they are positioned. However, Butler never offered a developmental mechanism to explain the establishment of these primordia, and he acknowledged that his theory lacked experimental evidence. Yet the notion of dental fields remained attractive (Colyer, 1991 [1936]). The rapid growth of molecular biology with the discovery of developmental fields and morphogen gradients may produce a modified form of Butler's theory (Goodwin, 1982, 1995; De Robertis, 1991; Gilbert *et al.*, 1996)

Edmund's (1960, 1962, 1969) Zahnreihen Theory proposed that tooth initiation is the result of a morphogen travelling from the front of the jaw quadrant to the back, inducing successive primordia at regular intervals. This model was constructed on descriptive evidence from tooth replacement patterns in both extant and fossil reptilian jaws and Edmund's interpretation of Woerdeman's (1919, 1921) embryological study of *Crocodylus porosus*. However, embryonic dental initiation patterns in fish (Berkovitz, 1977a, 1977b, 1978), frogs (Shaw, 1979, 1982, 1986; Chapters 2 and 3) and reptiles (Osborn, 1971; Westergaard, 1988a, 1988b; Westergaard and Ferguson, 1986, 1987, 1990) are not consistent with the patterning predicted by the Zahnreihen theory. Osborn (1971), Westergaard and Ferguson (1987) and Westergaard (1988a) also demonstrated how Edmund misinterpreted Woerdeman's embryological findings. Moreover, evidence of morphogens running the length of the jaw does not exist. However, the influence of this model still remains (Keiser *et al.*, 1993).

Osborn (1971, 1973, 1978, 1984, 1993) proposed that a clone or discrete group of ectomesenchymal cells expands in the jaw quadrant during development, giving rise to the entire dentition in reptiles. In mammals three clones exist--a posteriorly growing incisive clone, a single canine clone, and a molar clone which grows both posteriorly and anteriorly. The Clone Theory asserts that dental initiation and dental shape gradients are intrinsic to the clone. A zone of inhibition surrounds the developing tooth which ensures not only the even spacing of teeth, but also accounts for tooth wave replacement in polyphyodonts. This model gained some experimental support when Lumsden (1978, 1979) transplanted murine presumptive M1 tissue (E12--dental lamina stage) intraocularly and an entire and recognizable molar series of three teeth developed. However, Lumsden did not elaborate on the nature of a clone; i.e., whether it was a "two-layer structure" composed of epithelial and mesenchymal cells or only mesenchymal cells.

Westergaard and Ferguson (1986, 1987, 1990) investigated embryonic dental initiation patterns in *Alligator mississipiensis* and proposed the New Progress Zone Model of the development of the dentition. Incorporating the notion that dental patterning is first established in the oral epithelium (Mina and Kollar, 1987), they speculated that once initiation occurs in the epithelium at the first dental locus, this "early initiation stimulus" is coupled with an "inhibition process" and spreads in the epithelium, resulting in zones of competent epithelium that induce the overlying mesenchyme into odontogenesis. This thesis also envisions a proliferative epithelial "progress zone" that grows in concert with the jaw growth, and that with either a cell lineage phenomenon and/or positional information phenomenon epithelial cell division ceases, giving rise to new dental anlagen.

Chapters 2 and 3 recorded the development of the early dentition longitudinally in larval *Xenopus laevis* between Stages 54 and 61. The dental initiation patterns note were not consistent with the previous development models of the dentition. Contrary to the Zahnreihen Theory, the order of initiation in tadpoles was irregular with varying interstitial tooth development and not sequential from the front of the jaw quadrant to back as predicted by Edmund. The Clone and New Progress Zone models predict that dentition pattern development is intimately related to jaw growth, but the tadpole quickly develops an initial dental row (IDR) of four to eight tooth germs (average length: 1136 μm) over a few days with little to no corresponding jaw growth (average growth: 71 μm). Moreover, both these models suggest that the dentition develops from an identifiable first dental locus (Osborn's term: "a clone determinant"). However, in the tadpole the first tooth in the mouth may appear anywhere between tooth positions 3 to 19, and coincidental initiations (up to 4 tooth germs) on the first day occur in half of the jaw quadrants. Chapter 3 then proposed the Odontogenic Field Theory. Dental development begins in the medial two-thirds of the jaw quadrant with the rapid appearance of an epithelial odontogenic field (OF) within which individual dental initiation zones (IZs) are competitively established. Dental initiation is intrinsically and locally controlled, proceeding once a critical volume of OF epithelium is established to create an IZ. A tooth germ then develops in the middle of an IZ. The odontogenic field later extends distally and palatally, adding teeth to the end of the dental row and between the first teeth initiated in the mouth.

Previous theories on the development of the early dentition have been based exclusively on static descriptive investigations [other than Lumsden's (1978, 1979) contribution]. In the present study, extirpations of the presumptive dental tissues from larval *X. laevis* were performed in order to investigate the nature of the mechanism that establishes this dentition. The transparency of these animals between Stages 40 and 61 and the horizontal orientation of their developing tooth germs permits surgical manipulation of the presumptive dental row and daily recording of the impact that an experimental procedure has on the developing dentition.

B. MATERIALS AND METHODS

Animals were obtained by induced breeding with human chorionic gonadotropin (HCG, Sigma Chemical Co., St. Louis, MO). At 4 PM adult females were injected with 800 IU of HCG and placed overnight with adult males which had received 400 IU of HCG at the same time. Embryos developed in gently aerated tap water treated with 1% sodium thiosulphate (2 mL per liter) and maintained at room temperature. At 3 days post-fertilization, the animals were transferred to 40 L stock tanks (approximately 200 animals/tank) featuring a one way constant flow (20 L/hr) of filtered (by activated carbon) and dechlorinated (treated with sodium thiosulphate) water held at a constant pH (7.8) and constant temperature (20 ± 0.5 C°). The animals were fed Tadpole Powder (Nasco, Fort Atkinson, WI) sprinkled over the water surface daily at 7AM and 6PM. Two varieties of snails aided in maintaining the cleanliness of the tanks. Excess detritus was removed biweekly. A 12h photo period was maintained (7 AM to 7 PM).

Tadpoles were separated from the stock population at the appropriate stage (Nieuwkoop and Faber, 1967) for surgical extirpation. They were anesthetized with 2% aminobenzoic acid ethyl ester (MS 222, Sigma Chemical Co., St. Louis, MO) and their mouths were opened with the aid of a retractor fabricated from orthodontic ligature wire. Chemically sharpened tungsten needles and corneal scissors were employed in surgeries. Experimental animals were placed into 10 L brown buckets that featured the aquatic conditions previously described. Small 1 mm incisions in the dorsal epithelium served to identify the tadpoles. A graticule mounted on the microscope was employed to measure the distance between developing tooth germs. In order to establish a spatial marker, all measurements were made from the most medial tooth germ. Interdental distances were recorded daily until Stage 61 when dramatic metamorphic changes in the head morphology rendered accurate recording impossible. The animals were sacrificed by anesthetic overdose. The dental row was stripped from the underlying suprarostral cartilage and analyzed under the culture microscope to establish the final tooth count. For histological analysis, the tissue was fixed in formalin, demineralized with a formic acid and sodium citrate solution, sectioned at 10 μm and stained with haematoxylin and eosin.

The terminology employed in Chapter 2 to describe the development of the early dentition development in larval *X. laevis* is used in this chapter. 'Primary tooth germs' are defined as the first tooth germs to appear in the dental row. Dental development begins quickly over 2 to 4 days during late Stage 54 in the medial two-thirds of the jaw quadrant with a row of 4 to 8 primaries (Initial Dental Row--IDR). These are followed by the distal and sequential addition of more primaries (Distal Dental Row--DDR) over the next 2-3 weeks. In most cases 1-3 tooth germs develop between the primaries at a more palatal position. A 'secondary tooth germ' develops between two primaries, and together these three tooth germs constitute the 'solitary configuration'. [Note: The dental terms 'mesial' and 'distal' are employed in this paper to refer to the 'medial' and 'lateral' aspects of the dental row in each jaw quadrant, respectively (Bitgood and McMahon, 1995; Mina *et al.*, 1995). 'Labial' means 'toward the lip' while 'palatal' indicates toward the palate.']. Three experiments were performed:

(1) MIDDLE THIRD JAW QUADRANT EXTIRPATION

The entire middle third of the upper right jaw was extirpated in 6 tadpoles at Stage 45 (10-11 mm) about three weeks prior to any histological evidence for the beginning of odontogenesis (Late Stage 54; mean: 27.8 DPF). The surgery was extended toward the border of the olfactory organ and included the oral tissues, the supporting suprarostral cartilage and the dorsal mesenchyme and epithelium (**Plate 5-1A**). The wound was monitored in order that tissue repair did not bridge the mesial and distal thirds of the jaw together. In all 6 animals it was necessary to freshen the wound four times during the observation period. The development of the dentition on the operated quadrant was recorded daily from late Stage 54 to 61 and measurements were made from the mesial-most tooth germ.

(2) MESIAL, MIDDLE AND DISTAL THIRD PRESUMPTIVE DENTAL ROW EXTIRPATIONS

Twenty-four animals were selected at Stage 53 (35-40 mm) and divided into three groups. A 250-350 µm section of presumptive dental row tissue that included the oral epithelium and mesenchyme up to the suprarostral cartilage was excised from one of three sites near the lip in the upper right jaw: (1) Tissue from the mesial third site included a margin cut along the midline (**Figure 5-1, Site A**). (2) In the middle third site, the section was extirpated from the center of that region (**Figure 5-1, Site B**). (3) Presumptive dental row from the distal third extended to the tentacle (**Figure 5-1, Site C**). Excised tissue was transplanted to a dorsal site between the olfactory nerves in the animal from which it was taken. A longitudinal incision through the dorsal epithelium was made between the olfactory bulbs. The epithelium was retracted laterally opening a bed of mesenchymal tissue. The mesenchymal surface of the graft was apposed to the dorsal mesenchyme, and the edges of the graft tucked under the dorsal epithelium (**Plate 5-1B**). As a result, the oral epithelium of the graft remained exposed and it was possible to record dental development during the recording period. Dental row development in the operated quadrant was recorded daily and measurements were made from the mesial-most tooth germ.

(3) COMPLETE PRESUMPTIVE DENTAL ROW EXTIRPATIONS

The entire presumptive dental row of the right upper jaw was excised from 12 Stage 53 (35-40 mm) tadpoles. The margins of the wound extended along the lip line between the midline and the tentacle, and the posterior margin was anterior to the olfactory bulb (**Figure 5-2**). All tissue up to the suprarostral cartilage was included. Dental development in the operated quadrant was recorded daily and measurements were made from the mesial-most tooth germ. Control animals received the identical surgery and pairs were sacrificed between Stages 53-59 for histological analysis.

The dental initiation patterns in these three experiments were compared to those recorded during normal development of the dentition in *X. laevis* tadpoles (Chapter 2). That study was based on 22 animals which were reared under the identical aquatic conditions in the present study.

C. RESULTS

(1) MIDDLE THIRD JAW QUADRANT EXTIRPATIONS

The development of the dentition pattern in the mesial and distal segments of the operated jaw was normal, and the animals developed at a standard rate and to a standard size. In the mesial segment, the primary tooth germs emerged suddenly between late Stage 54 and early Stage 55 in a manner similar to the IDR in normal development. In the distal jaw segment, the dental row extended back from the wound site during the development of the dentition. Primary tooth germs appeared sequentially at alternate tooth positions, a dental initiation pattern consistent with the DDR in normal development. Both mesial and distal jaw segments featured alternation between even-numbered and odd-numbered tooth positions with the former tooth germs appearing before the latter, resulting in the characteristic even-type (ET) dental pattern of this tadpole (Shaw, 1979; Chapter 2).

One animal died under the anesthetic at Stage 56. The other five animals survived to Stage 61 and developed an average of 12.2 tooth germs (range: 11-13 tooth germs). The wound site in one animal was skewed toward the midline and the mesial segment had only 2 germs while the distal segment contained 11 tooth germs. The dental pattern of this latter segment featured the characteristic sloping of the DDR. The other four animals had the surgical defect in approximately the middle of the jaw quadrant. In these tadpoles, the mesial jaw segment developed an average of 7 tooth germs (range: 6-8), and the distal jaw segment averaged 5 tooth germs (range: 3-7). **Figure 5-3** presents the average tooth germ occupancy time at each tooth position for these 4 animals. The resultant pattern reflects that in normal development (Chapter 2, **Figure 2-2**) less the tooth germs in the middle third of the jaw quadrant.

(2) MESIAL, MIDDLE AND DISTAL THIRD PRESUMPTIVE DENTAL ROW EXTIRPATIONS

Dental development was noted in all the recoverable grafts of the presumptive dental row regardless of their site of origin in the jaw quadrant (**Plate 5-2**). Four grafts were rejected soon after the surgery at a stage prior to the beginning of normal dental development (between late Stage 54 and Stage 55). The average tooth germ occupancy time in the grafts from the mesial, middle and distal thirds of the jaw was nearly identical (11.7, 11.6 and 11.5 days, respectively). **Figures 5-4, 5-5** and **5-6** present the average tooth germ occupancy time of only the primary tooth germs in both the jaw quadrant and the graft for the three surgical groups. All three experimental patterns reveal that the effect of the extirpation extended beyond the borders of the surgical site as compared to normal development (Chapter 2, **Figure 2-2**). For example, removal of the distal third tissue reduced the occupancy time of the primaries in the middle third of the jaw. This observation was confirmed in the individual dental development records. However, in the mesial and middle third extirpations, the developmental pattern in the distal third was quite similar to that in the non-surgical animals. That is, it reflected the sloping DDR of primary tooth germs. Finally, the first tooth germs that appeared in the surgical sites failed to show sequential or directional order.

(3) COMPLETE PRESUMPTIVE DENTAL ROW EXTIRPATIONS

The dental development patterns of animals that had the complete presumptive dental row extirpated at Stage 53 did not have the characteristic IDR plateau and DDR slope. Instead, an initial regeneration dental row (IRDR) of primary tooth germs was quickly established across the entire jaw quadrant. The average occupancy time of IRDR primaries at all tooth positions was about 5 days (**Figure 5-7**).

Applying the criterion employed to identify the IDR in normal development (that all tooth germs which appear in the first four days of the development of the dentition belong to this dental row; Chapter 2), the IRDR contained an average 9.9 tooth germs (range: 8-12 tooth germs) and was 2252 μm long (range: 1750-2450 μm, SD: 196). The IRDR was established in an average of 3.1 days during which the jaw quadrant grew 23 μm (range: 0-75 μm). At the end of the recording period, the final regenerative dental row

(FRDR) featured an average 14.7 tooth germs (range: 8-19 tooth germs) and was 2315 µm in length (range: 1750-2750 µm, SD: 235). The average interdental distance between the tooth germs in the IRDR at initiation was 240 µm (range: 125-500 µm, SD: 72). The mean interdental distance was 213 µm (SD: 37) in those interdental sites which had the characteristic initiation width of the solitary configuration in normal development.

Histologically, the surgical site at Stages 54-55 featured an irregular epithelium covering the wound. By Stage 59 this cartilage was fully regenerated and a characteristic zone of mesenchymal tissue was present between it and a regular oral epithelium. Normal tooth germs were found developing in this mesenchymal zone.

D. DISCUSSION

(1) RECONSIDERING MODELS FOR THE DEVELOPMENT OF THE DENTITION

The longitudinal study of the development of the early dentition in larval *X. laevis* revealed that the dental initiation patterns in the tadpole were not consistent with that expected by any of the standard theories on the development of the dentition--Zahnreihen, Clone or New Progress Zone (Chapter 2). The experimental extirpations of this developing dentition further support this contention.

Two experiments were designed to investigate Edmund's (1960, 1962, 1969) theory that a morphogen travels from the front of the jaw to the back initiating teeth along its path. In the first, the middle third of the jaw was excised (Stage 45; 6 DPF) about three weeks before any histological evidence of the commencement of odontogenesis. That is, the path was removed along which the purported morphogens travel from a midline morphogen field generator to the back of the jaw. However, tooth germs developed in the distal segment, making the existence of a traveling initiatory morphogen unlikely. For that matter, the dental development pattern found in the distal segment was typical of that in the DDR of nonsurgical animals. In a second extirpation experiment, presumptive dental row tissue from the distal third of the jaw at Stage 53 was transplanted outside the mouth to the dorsal surface and away from the theoretical field generator. This tissue was excised 4 to 5 days prior to the first histological evidence of odontogenesis in the mesial two-thirds of the jaw. Dental development occurred in all the recoverable grafts. That is, dental initiation in the graft arose independently of the purported oral morphogen.

These two experiments were also employed to test Osborn's (1971, 1973, 1978, 1984, 1993) claim that the dentition originates from a growing clone of cells. Both assumed Osborn's suggestion that lower tetrapods have only 1 clone in each jaw quadrant from which that quadrant's dentition originates. As a result, it is reasonable to assume that the clone determinant is organized somewhere in the mesial two-thirds of the tadpole jaw since this is the region where dental development begins (Chapter 2). In the middle third jaw extirpation (Stage 45) experiment, the presumptive clone determinant was either removed in the surgery or it was missed and remained in the mesial third of the jaw. As a result, two outcomes were possible. In the former, no dental development was expected. In the latter, tooth germs would appear only in the mesial segment. In both situations, dental development was not predicted in the distal segment. However, in every case tooth germs were found in both the mesial and distal jaw segments of all the experimental animals. In the transplantation of distal third presumptive dental tissue to the dorsal surface (Stage 53), the graft should not contain any dental clone tissue since the clone determinant is in the mesial two-thirds of the jaw. However, dental development occurred in all recoverable grafts. These two experiments demonstrate the independence of the development of the dentition from a specific site or clone determinant in the jaw.

The New Progress Zone Theory (Westergaard and Ferguson, 1986, 1987, 1990) features elements of both the Zahnreihen and Clone theories. Dental initiation begins in the epithelium near the front of the jaw (Tooth Position 3 in the alligator) and an "early initiation stimulus" then spreads from this site through the epithelium. The epithelium is also envisioned as a proliferative "progress zone" that grows in concert with the jaw growth. However, the experimental results in this paper that are inconsistent with travelling signals in the jaw or a growing zone of cells accounting for the entire dentition also render the New Progress Zone theory unlikely.

It must be emphasized that the most important models describing the development of the dentition were constructed from histological data. The experimental data in this study was performed at this level of investigation and failed to support these theories. Thus, evidence is necessary to establish the possible existence of travelling morphogens, clones or progress zones at another biological level and their operation prior to Stage 45 in *X. laevis*.

(2) THE ODONTOGENIC FIELD THEORY

The Odontogenic Field Theory proposes that an epithelial odontogenic field (OF) quickly develops in the mouth and that individual dental initiation zones (IZs) are competitively established within it (Chapter 3). Once a critical mass of OF epithelial cells is established, an IZ is created and a tooth germ autonomously emerges in its center. The

IZs also act as inhibitory zones restricting the initiation of other tooth germs within them while active. As a result, they are instrumental in determining the spacing between tooth germs and ultimately the pattern of the dentition. The IZs later recede freeing up OF epithelium for the establishment of new IZs between the first tooth germs. The embryonic dental initiation patterns recorded longtitudinally in larval *X. laevis* indicate that initiation is controlled locally (Chapters 2 and 3). This assertion was confirmed experimentally when a tooth germ was extirpated early in the development of the dentition and the initiation pattern that emerged reflected the surgically reconstituted developmental context of the region (Chapter 4).

Three important features of the Odontogenic Field Theory were seen in the present study:

(1) Dental initiation is controlled locally and is not dependent on distant regions of the developing jaw and dental row as suggested by the standard theories on the development of the dentition. That is, the presumptive dental tissues achieved dental competence independently of the neighboring regions of the jaw. Moreover, the pattern of dental initiation in a region was specific to that region. Three lines of evidence support these contentions. First, extirpations of the middle third of the jaw at Stage 45 (6 DPF) saw normal dental initiation patterns in the mesial and distal jaw segments. Second, recoverable grafts of the presumptive dental row to an extra-oral site all developed tooth germs.

Finally, in animals that received either a mesial or middle third presumptive dental row extirpation, dental development in the distal third of the jaw quadrant was characteristic of the normal DDR.

(2) The development of the dentition is not directly dependent on jaw growth, but begins with the rapid establishment of an epithelial odontogenic field across the jaw. Extirpation of the presumptive complete dental row resulted in the emergence of a row of primary tooth germs (IRDR) that resembled strikingly the IDR in normal development. The average times to establish these initial dental rows were nearly identical and the amount of jaw growth during this period was minimal (IRDR: 3.1 days, 23 μm; IDR: 3.2 days, 71 μm). However, the average row length in each of these dental rows far exceeded the jaw growth during the time of their establishment (IRDR: 2252 μm. IDR: 1137 μm).

(3) Dental initiation patterns reflect competition between IZs prior to dental morphogenesis. Two lines of evidence support this assertion. First, in the partial and complete presumptive dental row extirpations, the first tooth germs initiated in the surgical sites appeared non-sequentially with no directionality (e.g., front-to-back). Second, it appears that the developmental dynamic in the IRDR and IDR are similar. In normal development, the average distance between primary tooth germs in the IDR was 242 μm (range: 125-450 μm). However, it was assumed that the interdental distance between primaries of the solitary configuration (218 μm) more accurately represented the actual width of the IZ (Chapter 3). That is, this configuration most likely reflects the butting of adjacent IZ margins while doublet and triad configurations probably have a zone of free OF tissue between the IZ borders of their primaries. In the IRDR the average interdental distance at initiation between primary tooth germs (240 μm) was nearly identical to that

found in the normally developing IDR. If only those initiation interdental dental distances characteristic of the solitary configuration are employed (125-275 μm), then the average interdental distance was 213 μm, suggesting that the average IZ width in the IDR and IRDR was nearly identical.

FIGURE 5-1. MESIAL, MIDDLE AND DISTAL THIRD PRESUMPTIVE DENTAL ROW EXTIRPATION

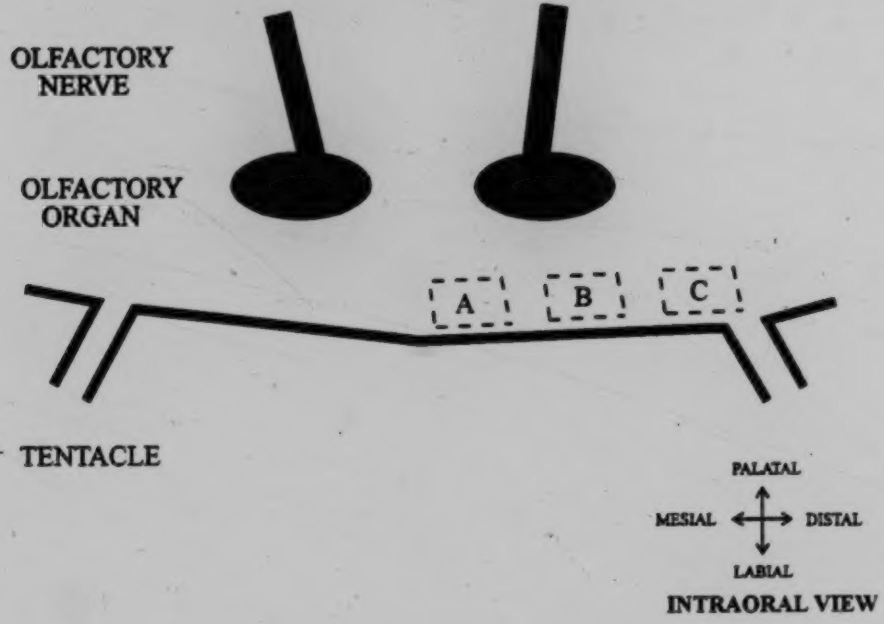
OLFACTORY NERVE
OLFACTORY ORGAN
A
B
C
TENTACLE
PALATAL
MESIAL
DISTAL
LABIAL
INTRAORAL VIEW

Figure 5-1. Mesial, Middle and Distal Third Presumptive Dental Row Extirpation. A schematic intraoral view of the three sites from which presumptive dental row tissue was excised at Stage 53. The surgery was performed at only one of the three sites in an experimental animal and the graft was transplanted to a dorsal site between the olfactory nerves. Dental development in both the surgical jaw quadrant and the graft was then recorded daily with a dissecting microscope.

FIGURE 5-2. COMPLETE PRESUMPTIVE DENTAL ROW EXTIRPATION

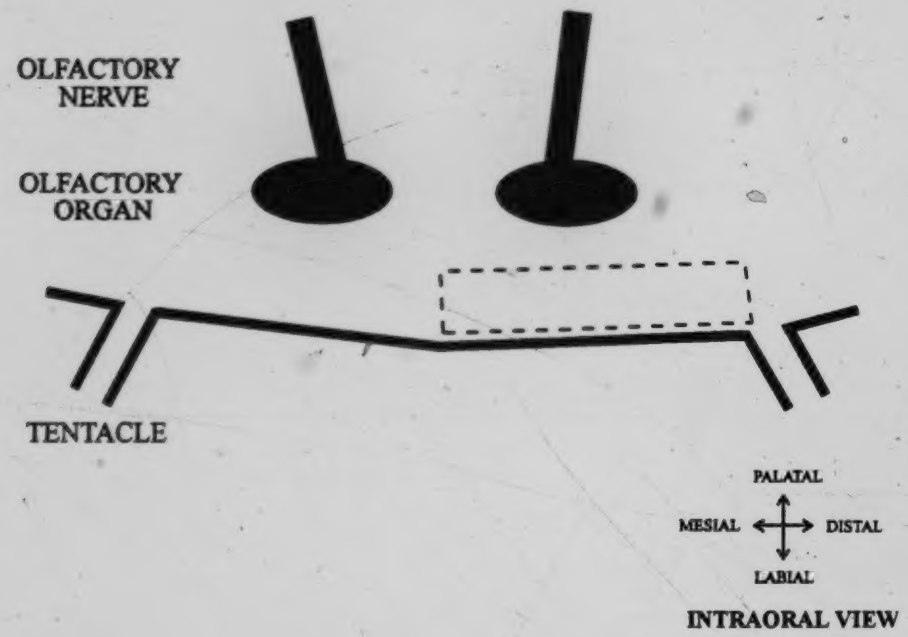
OLFACTORY NERVE
OLFACTORY ORGAN
TENTACLE
PALATAL
MESIAL
DISTAL
LABIAL
INTRAORAL VIEW

Figure 5-2. Complete Presumptive Dental Row Extirpation. A schematic intraoral view of the site from which presumptive dental row tissue was excised at Stage 53. The regeneration of the dental row in the surgical jaw quadrant was then recorded daily with a dissecting microscope.

FIGURE 5-3. MIDDLE THIRD JAW EXTIRPATION SURGERY:
GENERAL TOOTH GERM DEVELOPMENT PATTERN
AVERAGE OCCUPANCY TIME AT TOOTH POSITIONS
4 ANIMALS: RIGHT JAW QUADRANT

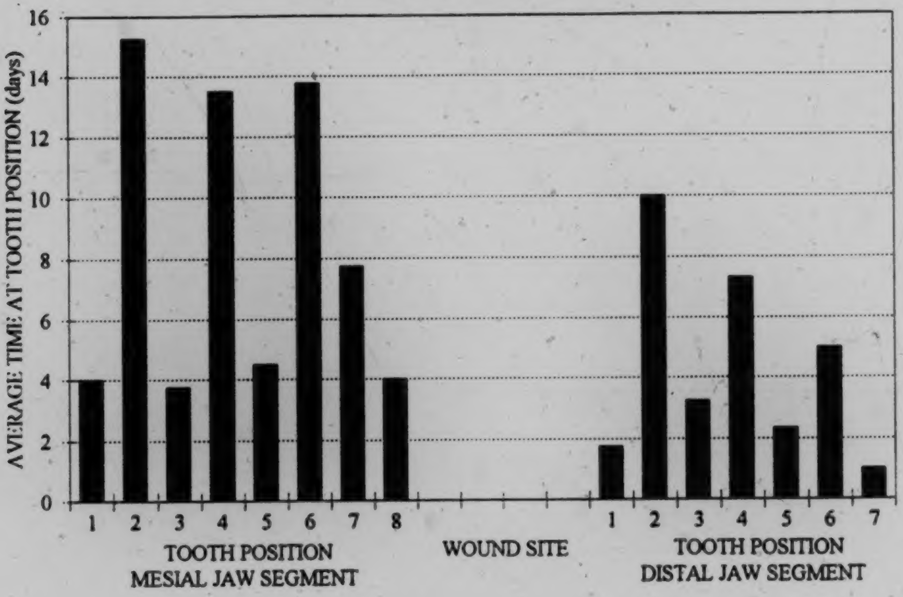

Figure 5-3. General Tooth Germ Development Pattern After Middle Third Jaw Extirpation. This composite shows the average number of days a tooth germ is present at every tooth position regardless of its developmental type during the odontogenic recording period (ORP; between Late Stage 54 and Stage 61). It shows a wound site that separates a "plateau" of the even-numbered tooth germs in the mesial segment of the jaw quadrant and a "slope" of these tooth germs in the distal segment.

FIG. 5-4. MESIAL THIRD PRESUMPTIVE DENTAL ROW EXTIRPATION: AVERAGE PRIMARY TOOTH GERM OCCUPANCY TIME IN THE DENTAL ROW AND THE GRAFT

8 ANIMALS: RIGHT QUADRANT

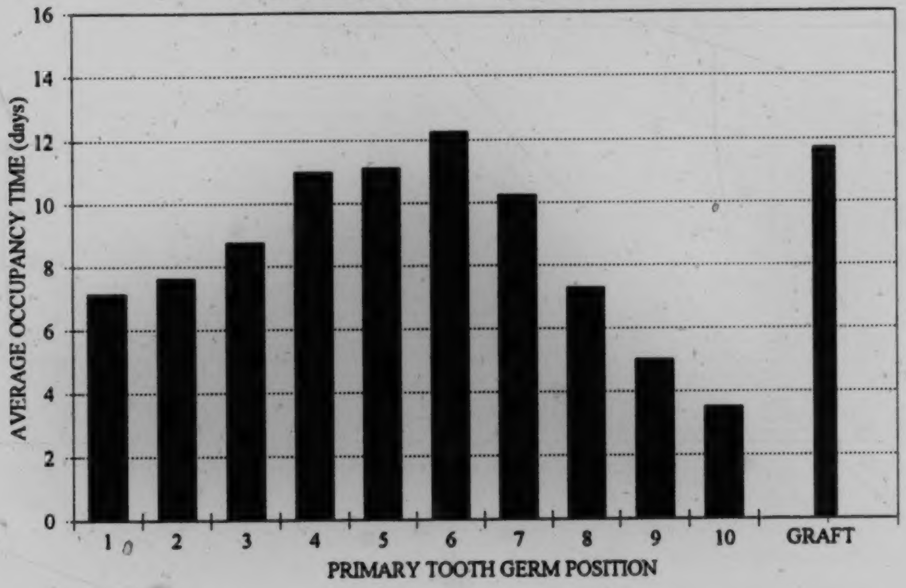

Figure 5-4. Primary Tooth Germ Development Pattern After Mesial Third Presumptive Dental Row Extirpation. This composite shows the average number of days a primary tooth germ was present in the experimental jaw quadrant during the odontogenic recording period (ORP; between Late Stage 54 and Stage 61). The position of the primaries in the quadrant is relative to only this tooth germ type and does not represent the final tooth position locus in the Complete Dental Row at Stage 61. The average number of days a tooth germ was present in the presumptive dental row tissue grafted to the dorsal site is represented by the bar at the right margin.

FIG. 5-5. MIDDLE THIRD PRESUMPTIVE DENTAL ROW EXTIRPATION
AVERAGE PRIMARY TOOTH GERM OCCUPANCY TIME
IN THE DENTAL ROW AND THE GRAFT
8 ANIMALS: RIGHT QUADRANT

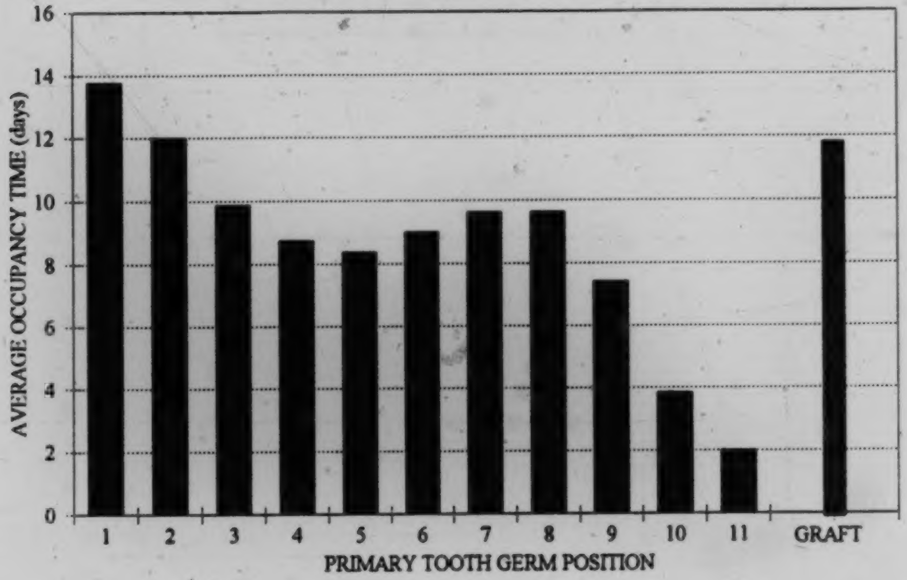

Figure 5-5. Primary Tooth Germ Development Pattern After Middle Third Presumptive Dental Row Extirpation. This composite shows the average number of days a primary tooth germ was present in the experimental jaw quadrant during the the odontogenic recording period (ORP; between Late Stage 54 and Stage 61). The position of the primaries in the quadrant is relative to only this tooth germ type and does not represent the final tooth position locus in the Complete Dental Row at Stage 61. The average number of days a tooth germ was present in the presumptive dental row tissue grafted to the dorsal site is represented by the bar at the right margin.

FIG. 5-6. DISTAL THIRD PRESUMPTIVE DENTAL ROW EXTIRPATION:
AVERAGE PRIMARY TOOTH GERM OCCUPANCY TIME
IN THE DENTAL ROW AND THE GRAFT

8 ANIMALS: RIGHT QUADRANT

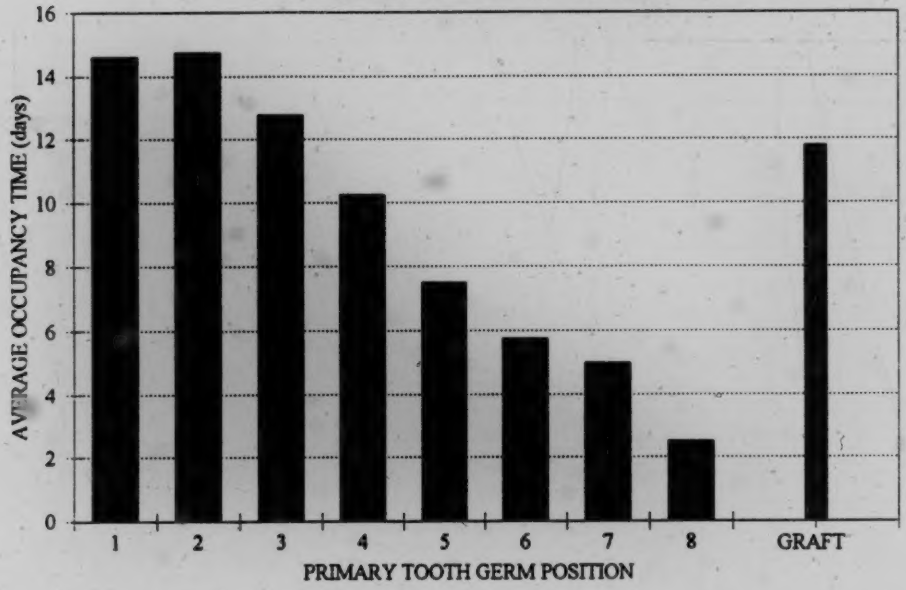

Figure 5-6. Primary Tooth Germ Development Pattern After Distal Third Presumptive Dental Row Extirpation. This composite shows the average number of days a primary tooth germ was present in the experimental jaw quadrant during the the odontogenic recording period (ORP; between Late Stage 54 and Stage 61). The position of the primaries in the quadrant is relative to only this tooth germ type and does not represent the final tooth position locus in the Complete Dental Row at Stage 61. The average number of days a tooth germ was present in the presumptive dental row tissue grafted to the dorsal site is represented by the bar at the right margin.

FIG. 5-7. COMPLETE PRESUMPTIVE DENTAL ROW EXTIRPATION: AVERAGE PRIMARY TOOTH GERM OCCUPANCY TIME

12 ANIMALS: RIGHT QUADRANT

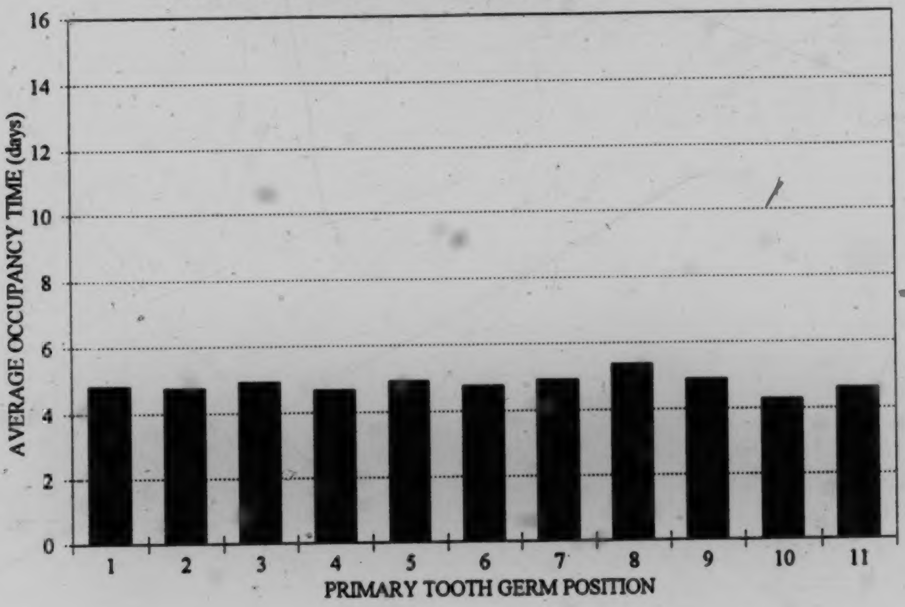

Figure 5-7. Primary Tooth Germ Development Pattern After Complete Presumptive Dental Row Extirpation. This composite shows the average number of days a primary tooth germ was present in the experimental jaw quadrant during the ORP (Late Stage 54 and Stage 61). The position of the primaries in the quadrant is relative to only this tooth germ type and does not represent the final tooth position locus in the Complete Dental Row at Stage 61.

Plate 5-1A. Dorsal surface of upper jaw immediately following surgical extirpation of the middle third of the upper jaw quadrant at Stage 45. Olfactory organ is the large dark pigmented structure associated with the external nares (E). Margins of upper lip (arrows). Labial is toward the right margin of the page. Unstained. Magnification (X 51).

Plate 5-1B. Intraoral graft positioned on dorsal surface of host immediately following surgery at Stage 53. Graft (arrow). External nares (asterisk). Labial is toward the bottom of the page. Unstained. Magnification (X 20).

PLATE 5-1

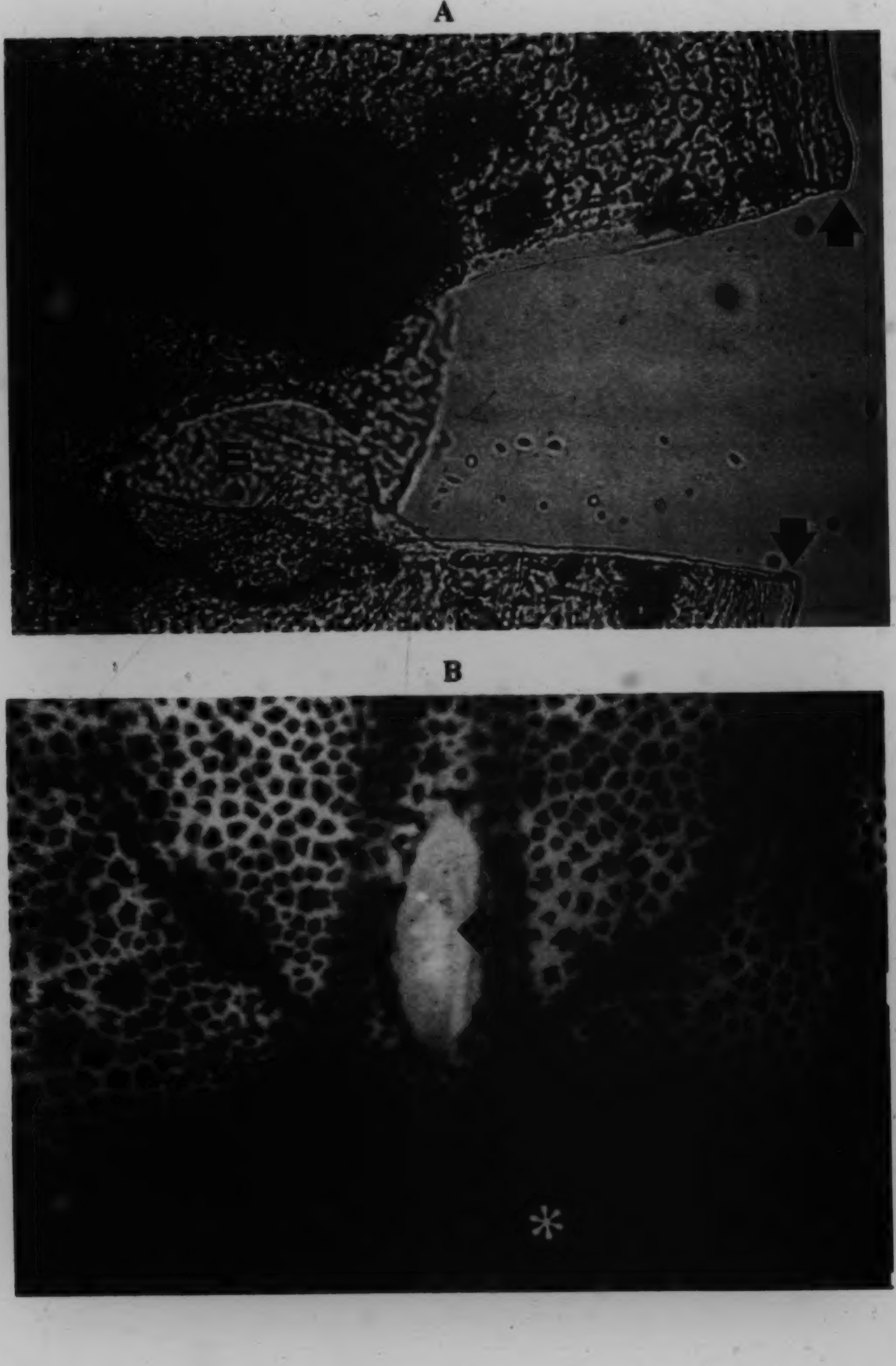

PLATE 5-2

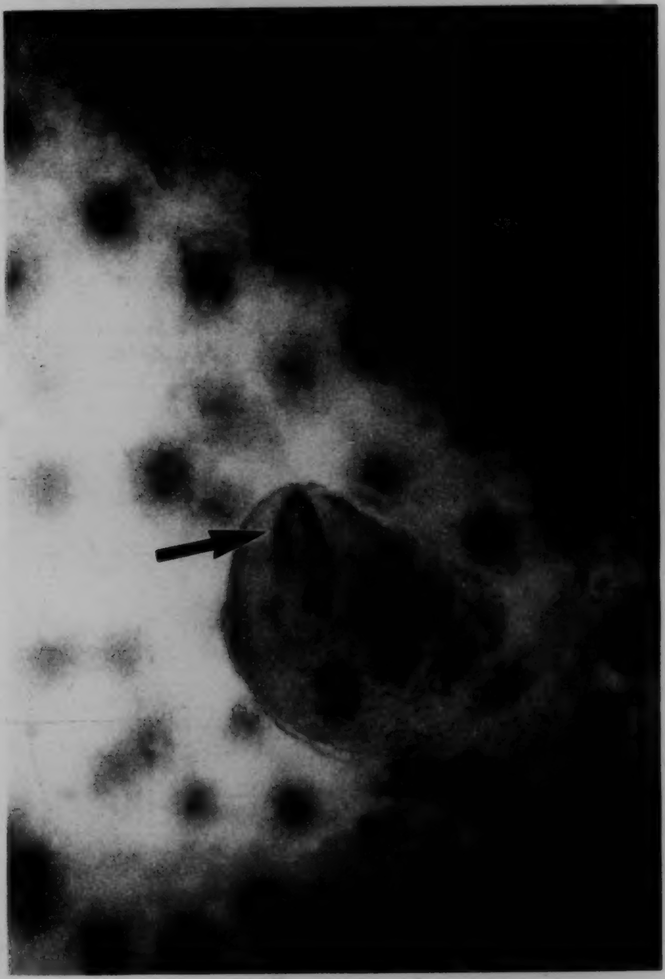

Plate 5-2. Intraoral graft attached to the dorsal surface of host (Stage 61). Developing tooth in graft (arrow). Labial is toward the bottom of the page. Unstained. Magnification (X 32).

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CHAPTER 6
DENTAL RECOMBINATION STUDIES IN XENOPUS LAEVIS (DAUDIN) AT STAGE 54

A. INTRODUCTION

An important theme in vertebrate development is the inductive interaction between cell layers in apposition that results in the morphologic conversion of one or both layers. Dental organogenesis provides a fine model for the investigation of these inductions in a complex sequence of reciprocal epithelial-mesenchymal interactions (Thesleff *et al.* 1995a, 1995b)

Earlier work on dental development revealed that heterotypic and heterospecific recombinations between dental mesenchyme and non-dental epithelium resulted in the production of teeth (Kollar and Baird, 1969, 1970a, 1970b; Thesleff, 1977; Thesleff *et al.*, 1991; Kollar and Fisher, 1980; Lemus *et al.*, 1986a, 1986b, Kollar and Mina, 1991). However, not all epithelia were capable of being instructively induced into an enamel organ (Kollar and Baird, 1970a).

Lumsden (1982, 1984, 1986, 1987, 1988) suggested that the initial odontogenic interaction was permissive and site specific. Influenced by Noden's (1983) thesis that neural crest cell populations acquire a regional morphogenetic specification prior to the onset of emigration, he wanted to determine whether the mesenchymal cells of murine teeth originated from a specified subpopulation in the neural folds. Lumsden first recombined premigratory cranial neural crest cells (E8) from the presumptive tooth-forming levels (i.e., the caudal mesencephalic and rostral metencephalic neural folds) and ectoderm from either the mandibular arch or limb (E9-E11). The former recombination resulted in molariform teeth with well-differentiated ameloblast and odontoblast cell layers juxtaposed to thin sheets of enamel and dentine. However, limb epithelium could not be induced into dental development. In another recombination, presumptive non-odontogenic neural crest from the trunk (E8) was combined with mandibular arch epithelium (E9) and teeth were produced. No teeth were formed with these neural crest cells in combination with limb epithelium (E9). Lumsden contended that murine neural crest cells have an odontogenic potential that is not limited to the tooth forming levels. Further, he asserted that cranial neural crest cells entering the mandibular arch are odontogenically uncommitted, and that in interacting with the mandibular epithelium this tissue layer is permissively induced to begin odontogenesis. Lumsden based his position on the fact that cranial neural crest cells failed to induce instructively limb epithelium into odontogenesis. As a result, Lumsden concluded that the oral epithelium is the earliest known site of dental pattern.

Mina and Kollar (1987) recovered molar-shaped teeth in isochronic recombinations of murine 1st branchial arch (mandibular) epithelium and 2nd branchial arch (hyoid) mesenchyme, underlining the instructive nature of the initial odontogenic induction. More specifically, they discovered that this inductive ability was limited only to early dental epithelium (E9-E11) since it was not present in older epithelium (E12+). In a reciprocal experiment, they recombined mandibular arch mesenchyme with hyoid arch epithelium and noted that odontogenesis occurred only in grafts performed after E12. In other words, it was only after the instructive induction of the mandibular mesenchyme by the mandibular epithelium that this mesenchyme was able to induce instructively the nondental epithelium.

Mina and Kollar concluded that the early oral epithelium initiates and organizes the dentition.

This study investigates the nature of the epithelial-mesenchymal interactions during early odontogenesis in *Xenopus laevis* larvae. Recombinations were made between oral and non-oral tissues, and these results were compared to murine studies in order to assess the evolutionary conservation of these interactions.

B. MATERIALS AND METHODS

Chapter 2 described the development of the early dentition longitudinally in larval *X. laevis*, and his terminology is employed in this paper. Dental development begins abruptly during late Stage 54 with a row of 4 to 8 tooth germs (Initial Dental Row--IDR). These are termed 'primaries' since they are the first tooth germs to appear in the dental row. More primaries are added sequentially at the back of the jaw (Distal Dental Row--DDR) over the next 2 to 3 weeks. Interdental tooth germs later develop between the primaries in both the IDR and DDR. A 'secondary' is the first tooth germ that develops between two primaries.

Animals were obtained by induced breeding with human chorionic gonadotropin (HCG, Sigma Chemical Co., St. Louis, MO). At 4 PM adult females were injected with 800 IU of HCG and placed overnight with adult males which had received 400 IU of HCG at the same time. Embryos developed in gently aerated tap water treated with 1% sodium thiosulphate (2 mL per liter) and maintained at room temperature. Once they were free-swimming tadpoles at 7 days post-fertilization, the animals were transferred to 40 L stock tanks (approximately 200 animals/tank) featuring a one way constant flow (20 L/hr) of filtered (by activated carbon) and dechlorinated (treated with sodium thiosulphate) water held at a constant pH (7.8) and constant temperature (20 ± 0.5 C°). The animals were fed Tadpole Powder (Nasco, Fort Atkinson, WI) sprinkled over the water surface daily at 7AM and 6PM. Two varieties of snails aided in maintaining the cleanliness of the tanks. Excess detritus was removed biweekly. A 12h photo period was maintained (7 AM to 7 PM).

Precocious tadpoles at Stage 54 (Nieuwkoop and Faber, 1967) were separated from the stock population. Animals were anesthetized with 2% aminobenzoic acid ethyl ester (MS 222, Sigma Chemical Co., St. Louis, MO) and their mouths were opened with the aid of a retractor fabricated from orthodontic ligature wire. Chemically sharpened tungsten needles and corneal scissors were employed to strip presumptive dental tissue from the underlying suprarostral cartilage (Trueb and Hanken, 1992). Specifically, 250-350 µm tissue segments were taken from the middle of the jaw quadrant in the area of the future Initial Dental Row (**Figure 6-1**). Nondental tissue of a similar size was taken from the dorsal surface between the olfactory nerves. Both dental and nondental tissues were transferred into Niu and Twitty medium (Rugh, 1962) and cooled to 4 C°. Tissue segments were then placed in 1% trypsin (Sigma Chemical Co. St. Louis, MO) for 2 hours at 4 C° and the epithelial and mesenchymal components were separated mechanically.

Tissue was taken at two different times: (1) early Stage 54 when there is no histological evidence of odontogenesis, and (2) three days later at late Stage 54 when the oral epithelium thickens at different sites in the IDR. All recombinations employed tissue from the same animal and the grafts were placed into the dorsal mesenchyme between the olfactory nerves of isochronic hosts. A recombination was performed using mandibular epithelium and dental mesenchyme at late Stage 54. Controls included transplanting early Stage 54 dental tissue after the epithelium and mesenchyme were separated enzymatically and then recombined. **Figure 6-2** schematizes the recombinations.

Each group of hosts was placed into 10 L brown buckets under the conditions previously described. The animals were monitored for 3 weeks and sacrificed by anesthetic overdose at Stage 61 when dramatic metamorphic changes began to occur in head morphology. The hosts were fixed in formalin, demineralized with a formic acid and sodium citrate solution, sectioned at 10 µm and stained with haematoxylin and eosin.

C. RESULTS

Table 6-1 outlines the results of the dental recombinations and controls that were grafted to the olfactory site of the host.

(1) EARLY STAGE 54

a. Oral Epithelium Only

Two-thirds of the grafts with only oral epithelium were completely rejected from the host. Those harvested have a dense epithelial ball rolled in on itself that is not integrated into the dorsal mesenchyme. One example has the graft has almost passed through the dorsal epithelium and is about to be rejected. There is no evidence of dental development at any graft site.

b. Oral Mesenchyme Only

Only one-third of the grafts were rejected from the host. The graft tissue is usually well-integrated into the host dorsal mesenchyme, but its distinctive pinkish staining pattern makes it possible to distinguish it from the host. There is no evidence of dental development.

c. Oral Epithelium and Dorsal Mesenchyme

Three of the five recovered grafts developed incipient dental organs with a thin dentine core. In all these grafts a second site of odontogenesis was noted with an epithelial dental organ in association with a dental papilla **(Plate 6-1A)**.

d. Oral Mesenchyme and Dorsal Epithelium

Nearly all these grafts were recovered. The graft epithelium and graft mesenchyme are distinct from the host and in almost every case these tissues are in good apposition. There is no evidence of dental development.

e. Oral Epithelium and Oral Mesenchyme Controls

All the controls of recombined early Stage 54 presumptive dental tissues developed a well-formed dental organ--a tooth, a dental papilla and dental epithelium **(Plate 6-1B)**. An enamel space is also present indicating the loss of enamel during the decalcification process. All the teeth harvested except one are similar to those in the normally developing dental row at the same stage of development **(Plate 6-2A)**.

(2) LATE STAGE 54

a. Oral Epithelium Only

Half of the grafts were rejected. The harvested grafts were similar to those containing only epithelium at early Stage 54. A dense epithelial ball often formed that did not integrate into the dorsal mesenchyme of the host. The rejection of the graft through dorsal epithelium is seen in some sections. There is no evidence of dental development at any graft site.

b. Oral Mesenchyme Only

Three of the four grafts recovered have sites of irregularly shaped dentinoid tissue in the host dorsal mesenchyme (**Plate 6-2B**). With the exception of one case, these hard tissues are not associated with any epithelial structures and are free in the dorsal mesenchyme. **Plate 6-3** shows an epithelial structure similar to a dental lamina extending from the dorsal epithelium to a small dentinoid mass.

c. Oral Epithelium and Dorsal Mesenchyme

All the grafts were recovered. Six of seven grafts have a definite dental papilla, an epithelial dental organ and an incipient tooth (**Plate 6-4A**). The exceptional graft is suggestive of the same. A narrow enamel space is present in many grafts, but it is not consistent in width. Some morphological distortion of the dentine is apparent with most of the teeth. Two grafts have a second site of odontogenesis with an epithelial dental organ and dental papilla, but no evident dentine.

d. Oral Mesenchyme and Dorsal Epithelium

This recombination offers the widest range of results. Two of the seven recovered grafts have a well-formed dental organ and a large tooth (**Plate 6-4B**). Two other grafts have small teeth. Another graft has only an epithelial dental organ. In five of the seven grafts, an epithelial dental organ or an epithelial structure similar to it is present.

e. Mandibular Oral Epithelium and Maxillary Oral Mesenchyme

All five grafts recovered feature irregular dentinoid deposits (**Plate 6-5**). There is no evidence of an epithelial dental organ in any of these. The epithelial graft appears as a dense ball similar to the epithelium only grafts. Those dentinoid structures in close apposition to the graft epithelium do not have an enamel space.

D. DISCUSSION

In larval *X. laevis*, presumptive dental tissues instructively induced nondental tissues into dental development. Dental epithelium at early or late Stage 54 recombined with isochronic dorsal mesenchyme from between the olfactory nerves produced recognizable teeth. Similarly, dental mesenchyme at late Stage 54 induced isochronic dorsal epithelium from this same region into tooth development.

Both early and late Stage 54 presumptive dental epithelium when grafted alone failed to instruct the surrounding host dorsal mesenchyme into odontogenesis. Once this epithelium was separated from its mesenchyme, the surface that had been apposed to the mesenchyme quickly rolled inward on itself and the graft formed a tight dense ball of epithelial cells. This suggests that dental epithelium is polarized and that only its mesenchymal surface has the ability to induce instructively nondental tissue into dental development. The external or exposed epithelial surface of these grafts did not integrate into the host mesenchyme, and this likely accounts for the high incidence of graft rejection (9/15).

Grafts containing only presumptive dental mesenchyme developed dentinoid tissue masses (3/4) if the enzymatic tissue separation from the epithelium had occurred at late Stage 54. The 6 grafts recovered of mesenchyme separated at early Stage 54 failed to produce hard tissue. This result is consistent with murine dental development where the mesenchyme requires an epithelial induction for the organization of the dental papilla and dentinogenesis to proceed (Mina and Kollar, 1987). The irregular shape of the dentinoid masses is most likely due to the lack of a definitive epithelial dental organ to direct morphogenesis. That is, once the oral mesenchyme was induced and then separated from its epithelial dental organ, dentinogenesis proceeded undirected in the host tissue. Interestingly, there is one example of an epithelial structure remarkably similar to a dental lamina extending from dorsal epithelium toward the oral mesenchymal graft (**Plate 6-3**). If this is a dental structure, then murine development is again reflected, since once a dental papilla is induced by the epithelium it reciprocates and instructively induces the overlying epithelium into dental lamina formation (Miller, 1969, 1971, Kollar and Baird, 1969, 1970a, 1970b; Kollar, 1981; Mina and Kollar, 1987; Kollar and Mina, 1991; MacKenzie *et al.*, 1991, 1992).

The instructive induction of the dorsal epithelium by induced oral mesenchyme was clearly seen in the recombination grafts of these tissues performed at late Stage 54. Four of seven grafts developed well-formed teeth, two of which were large. However, there was no evidence of odontogenesis (i.e., either soft or hard tissue) when these recombinations were made at early Stage 54. This inductive difference between heterochronic mesenchymal tissues is explicable in the light of murine dental development (Mina and Kollar, 1987). Similar to mouse oral mesenchyme at E9 to E11, early Stage 54 tadpole oral mesenchyme has yet to be instructively induced by the oral epithelium, and as a result, removing the mesenchyme prior to this time prevents its further participation in the epithelial-mesenchymal interactions of odontogenesis. However, by late Stage 54 (similar to E12+ murine mesenchyme) the oral epithelium has instructively induced the oral mesenchyme, and this latter tissue reciprocates by inducing the overlying oral epithelium into epithelial dental organ formation. This second induction is also instructive since it can induce nondental epithelium into odontogenesis. In the E12+ mouse, this mesenchyme can instruct both foot epithelium (Kollar and Baird, 1970b) and second branchial arch epithelium (Mina and Kollar, 1987) to form teeth. Similarly, late Stage 54 tadpole oral mesenchyme can induce dorsal epithelium into odontogenesis.

The recovery of dental tissues in recombinations of oral epithelium and dorsal mesenchyme at early Stage 54 suggests the instructive nature of the dental epithelium. This finding also mirrors murine dental development. In contrast to the well-formed teeth recovered in the controls performed at this stage (5/6), two of the three grafts featuring teeth only had small amounts of dentine deposited. This maybe due to the fact that technically this was the most difficult graft recombination. The epithelial tissue at the time of separation was very delicate and easily torn, and its apposition to dorsal mesenchyme was always problematic. As a result, it may be for technical and mechanical reasons that this recombination did not yield more large and fully formed teeth. However, a definitive epithelial dental organ and dental papilla were recovered in 3 of 4 grafts.

The results of the recombinations of oral epithelium and dorsal mesenchyme at late Stage 54 differs from predictions based on the murine dental model. In mouse tooth development the dental epithelium loses its ability to instructively induce the mesenchyme once it has done so by E12. However, this does not appear to be the case in *X. laevis* larvae since late Stage 54 oral epithelium can instruct dorsal mesenchyme into odontogenesis. This finding is explicable, though, in the light of the dental initiation patterns in the tadpole. About 8 days after the initiation of a row of primary tooth germs (IDR) at late Stage 54, secondary tooth germs are initiated between these first tooth germs (Chapter 2). The fact that the teeth recovered in recombinations of late Stage 54 oral epithelium and dorsal mesenchyme were clearly smaller than those found in both the controls and the grafts of late Stage 54 oral mesenchyme and dorsal epithelium suggests that these teeth are secondaries. That is, the instructive odontogenic induction of dorsal mesenchyme likely came from secondary tooth germ oral epithelium about one week after the initiation of the IDR primary tooth germs. In contrast, the teeth recovered in the controls and late Stage 54 recombinations were large because they are IDR primaries.

Impetus to recombine mandibular oral epithelium with maxillary oral mesenchyme was that at the beginning of odontogenesis this epithelium is tightly apposed to the underlying infrarostral and Meckel's cartilages. It was assumed that since the evolutionary precursor to this jaw once bore teeth, the loss of teeth may have been due to the limited amount of mesenchymal tissue between this epithelium and these cartilages. Grafting of late Stage 54 oral mesenchyme failed to induce the mandibular epithelium into a epithelial dental organ. It would seem that this tissue, like murine snout epithelium (Kollar and Baird, 1970b), has stabilized and cannot react to the instructive signal of an activated dental mesenchyme. Irregular masses of dentinoid tissue were present in all grafts. These were similar to grafts with only activated mesenchyme, suggesting the lack of any epithelial participation in the laying down of this tissue.

The similarities in the nature of dental epithelial-mesenchymal interactions between the tadpole and the mouse suggests the evolutionary conservation of the basic odontogenic mechanisms.

TABLE 6-1. DENTAL RECOMBINATION RESULTS

	Grafts Recovered	Grafts Lost/Rejected	Teeth/ Dentine/ Dentinoid	General Description	Interpretation
(1) EARLY STAGE 54					
a. Oral Epithelium Only	3	6	No	Dense epithelial balls not integrated into dorsal mesenchyme. In one specimen the graft is being rejected through dorsal epithelium.	Inner surface of epithelium rolls in on itself and graft never integrates into the dorsal mesenchyme.
b. Oral Mesenchyme Only	6	3	No	Graft usually well-integrated into dorsal mesenchyme, but it can be distinguished from host.	Oral mesenchyme has not been instructively induced by oral epithelium.
c. Oral Epithelium & Dorsal Mesenchyme	5	4	Yes 3/5 grafts	Incipient dental organs associated with a thin dentine layer. A second site of odontogenesis noted in all 3 successful grafts, but none have an associated tooth.	Oral epithelium instructively induced the dorsal mesenchyme into odontogenesis.
d. Oral Mesenchyme & Dorsal Epithelium	10	1	No	Graft mesenchyme and epithelium well associated and can be distinguished from host. Cystic degeneration often seen with graft epithelium.	Oral mesenchyme has not been instructively induced by oral epithelium and thus cannot instructively induce the epithelium.
e. Controls: Oral Mesenchyme & Oral Epithelium	5	0	Yes 5/5 grafts	Well-formed teeth found in all grafts.	Enzymatic separation did not inhibit normal dental development.
(2) LATE STAGE 54					
a. Oral Epithelium Only	3	3	No	Dense epithelial balls not intergrated into dorsal mesenchyme. Evidence in one specimen of rejection from host.	Instructively inducing inner surface of epithelium rolls in on itself and graft never integrates into the dorsal mesenchyme.
b. Oral Mesenchyme Only	4	1	Yes 3/4 grafts	Dentinoid mass without an associated epithelium. One example of an epithelial lamina extending from the dorsal surface to the dentinoid mass.	Mesenchyme instructed by oral epithelium before grafting. Odontoblasts secrete dentine in an irregular manner since there is no dental epithelial organ to guide morphogenesis.
c. Oral Epithelium & Dorsal Mesenchyme	7	0	Yes 6/7 grafts	Epithelial dental organ and incipient tooth. Two specimens have a second site of odontogenesis, but no associated tooth.	Oral epithelium of secondary tooth site initiates dorsal mesenchyme. The tooth is small because secondaries are initiated late in the ORP.
d. Oral Mesenchyme & Dorsal Epithelium	7	0	Yes 4/7 grafts	Dental epithelium associated with teeth that range from only a cusp tip to large and well-formed tooth.	Induced oral mesenchyme of a primary tooth instructs dorsal epithelium. The tooth is large because primaries are initiated early in the ORP.
e. Mandibular Oral Epithelium & Maxillary Oral Mesenchyme	5	0	Yes 5/5 grafts	Dentinoid (dentine?) issue present in all grafts, but there is no evidence of an epthelial organ.	Mesenchyme initiated by oral epithelium before grafting, and odontoblasts secrete dentinoid (dentine?) in an irregular manner. Mandibular epithelium already stabilized and cannot be induced into odontogenesis.

FIGURE 6-1. INTRAORAL SURGICAL SITE

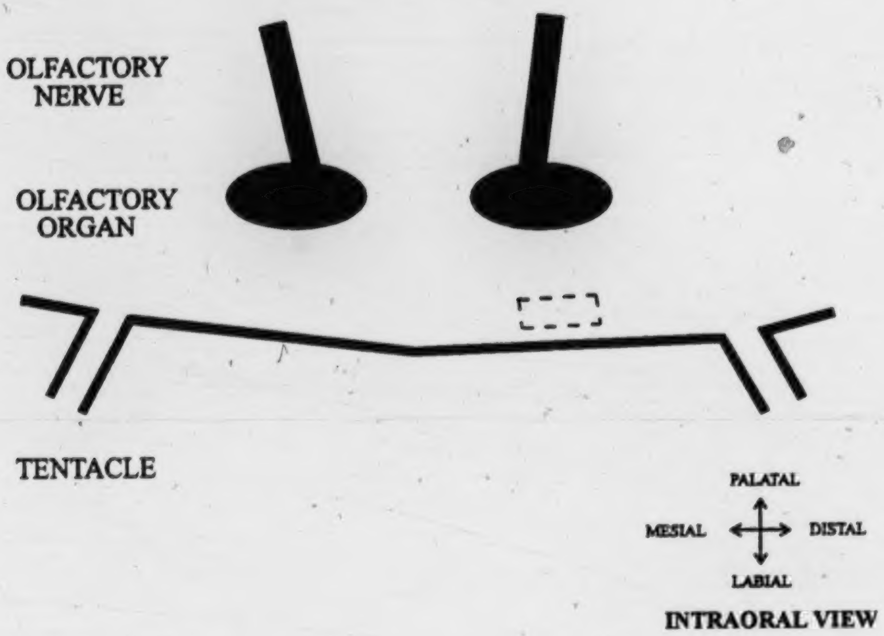

OLFACTORY NERVE
OLFACTORY ORGAN
TENTACLE
PALATAL
MESIAL
DISTAL
LABIAL
INTRAORAL VIEW

Figure 6-1. Intraoral Surgical Site. A schematic intraoral view of the site from which presumptive dental row tissue was excised between early and late Stage 54 for the dental recombination studies.

FIGURE 6-2. DENTAL RECOMBINATIONS

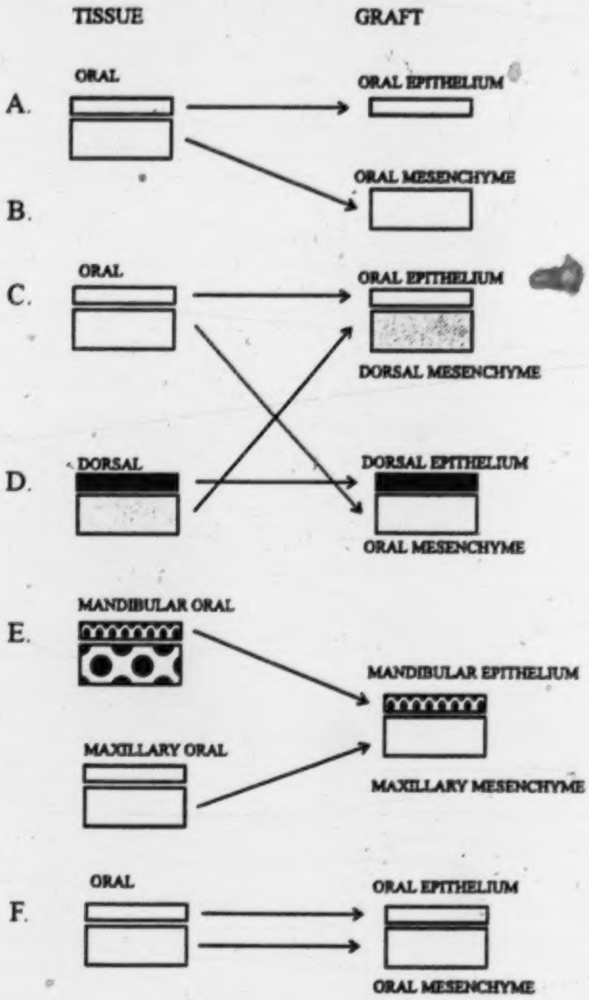

TISSUE
GRAFT
ORAL
A.
ORAL EPITHELIUM
ORAL MESENCHYME
B.
ORAL
C.
ORAL EPITHELIUM
DORSAL MESENCHYME
DORSAL
D.
DORSAL EPITHELIUM
ORAL MESENCHYME
MANDIBULAR ORAL
E.
MANDIBULAR EPITHELIUM
MAXILLARY ORAL
MAXILLARY MESENCHYME
ORAL
F.
ORAL EPITHELIUM
ORAL MESENCHYME

Figure 6-2. Dental Recombinations. The schematic diagram presents the presumptive dental tissues separated and their recombinations at both early and late Stage 54 (**A-D**). The recombination in **E** was performed at late Stage 54. The control grafts (**F**) were done at early Stage 54.

Plate 6-1A. Early Stage 54 oral epithelium and dorsal mesenchyme graft. Two well-defined germs tooth germs present. Slight dentine cusp tip (arrow) seen in right tooth germ. Dorsal surface is toward top of the page. Stained with haematoxylin and eosin. Magnification (X 128).

Plate 6-1B. Early Stage 54 oral epithelium and oral mesenchyme graft. Tooth germ with dentine wall of tooth (small arrows). Dental papilla (large arrow). Dorsal mesenchyme (M). Dorsal surface is toward top of the page. Stained with haematoxylin and eosin. Magnification (X 128).

PLATE 6-1

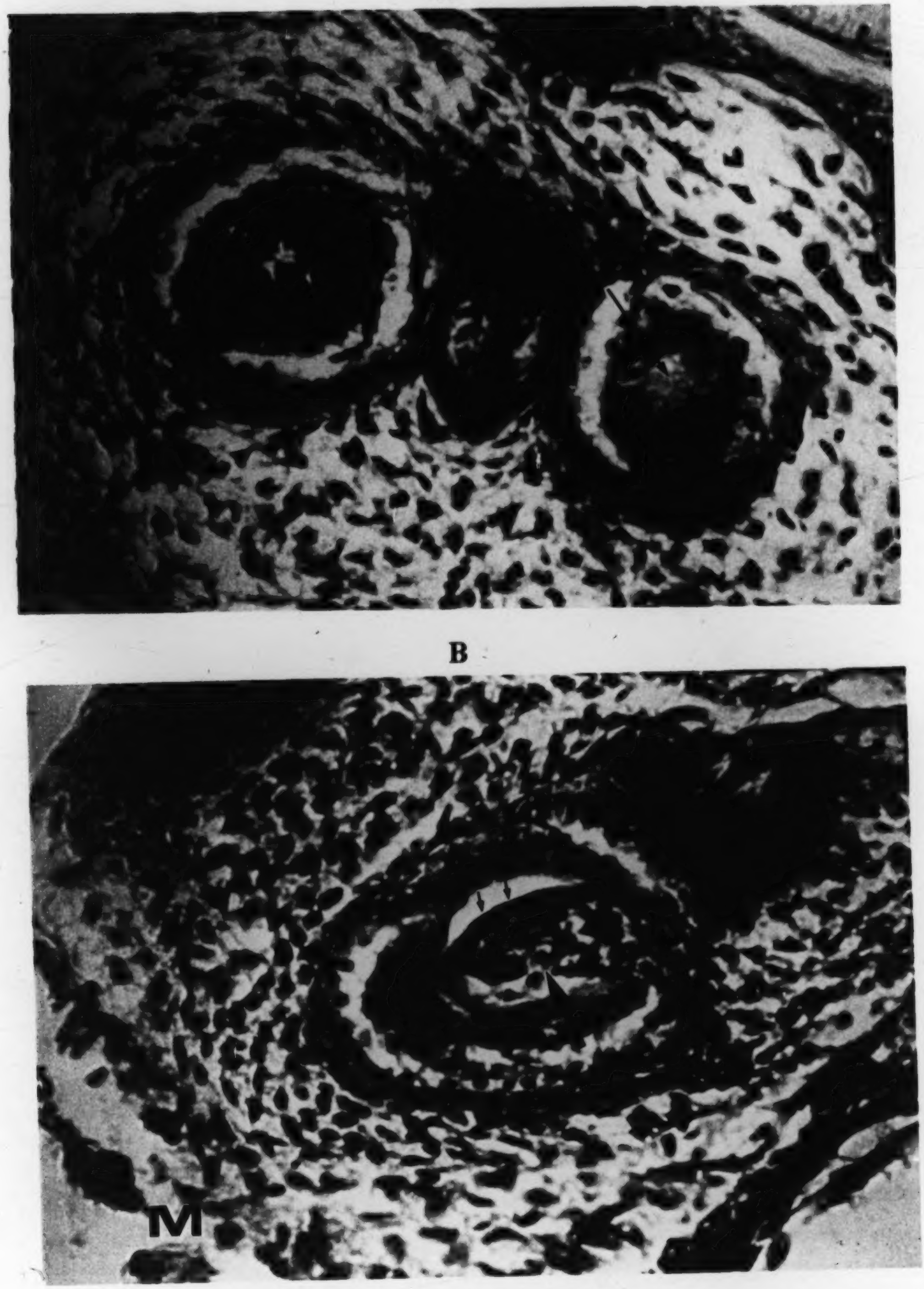
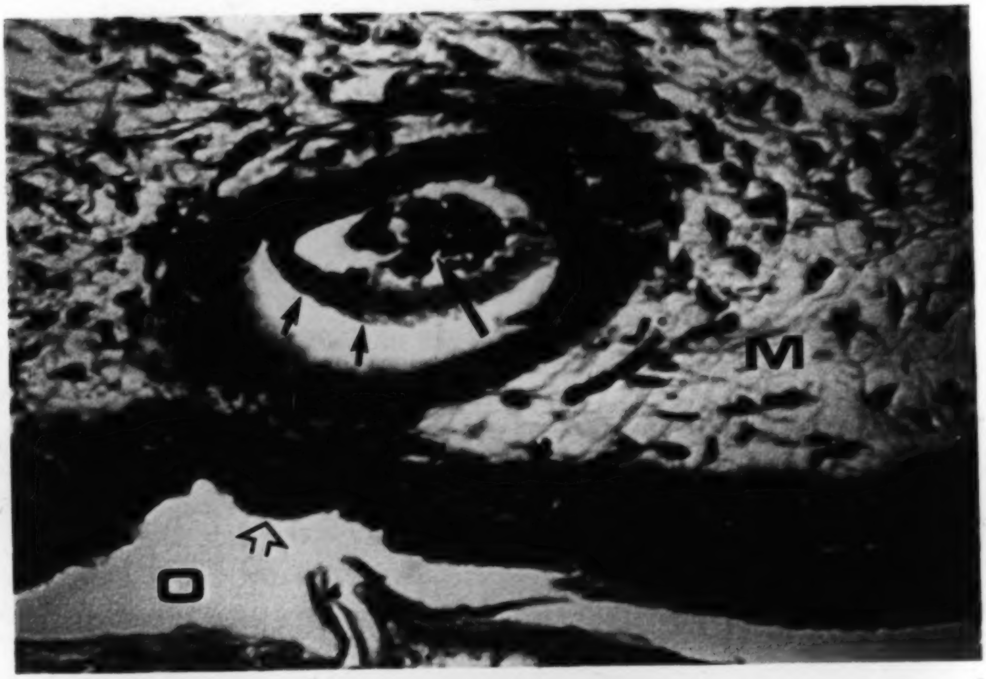


Plate 6-2A. Normal tooth germ of upper jaw of a control animal at Stage 59. Dentine wall of tooth (small arrows). Dental papilla (large arrow). Oral epithelium (open arrow). Oral mesenchyme (M). Oral cavity (O). Dorsal surface is toward top of the page. Stained with haematoxylin and eosin. Magnification (X 128).

Plate 6-2B. Late Stage 54 oral mesenchyme only graft produced irregular dentinoid tissue (arrows). Dorsal mesenchyme (M). Dorsal surface is toward top of the page. Stained with haematoxylin and eosin. Magnification (X 128).

PLATE 6-2

A



B

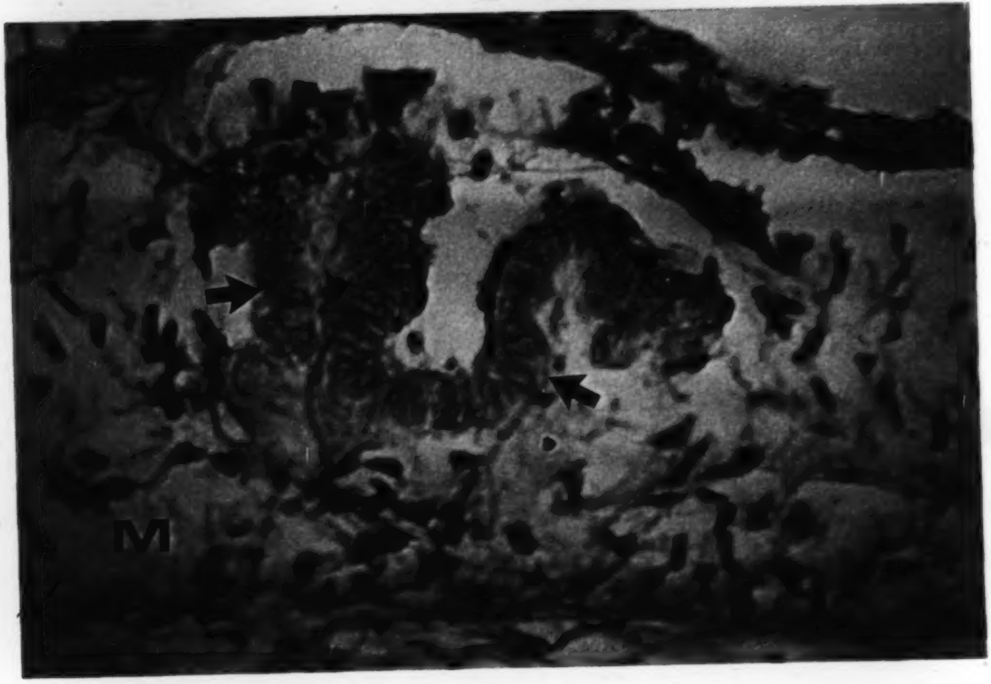


Plate 6-3. Late Stage 54 oral mesenchyme only graft. An epithelial structure extends from the dorsal epithelium (D) to a dentinoid mass (arrow). Dorsal mesenchyme (M). Suprarostral cartilage (C). Dorsal surface is toward the top of the page. Stained with haematoxylin and eosin. Magnification (X 128).

PLATE 6-3

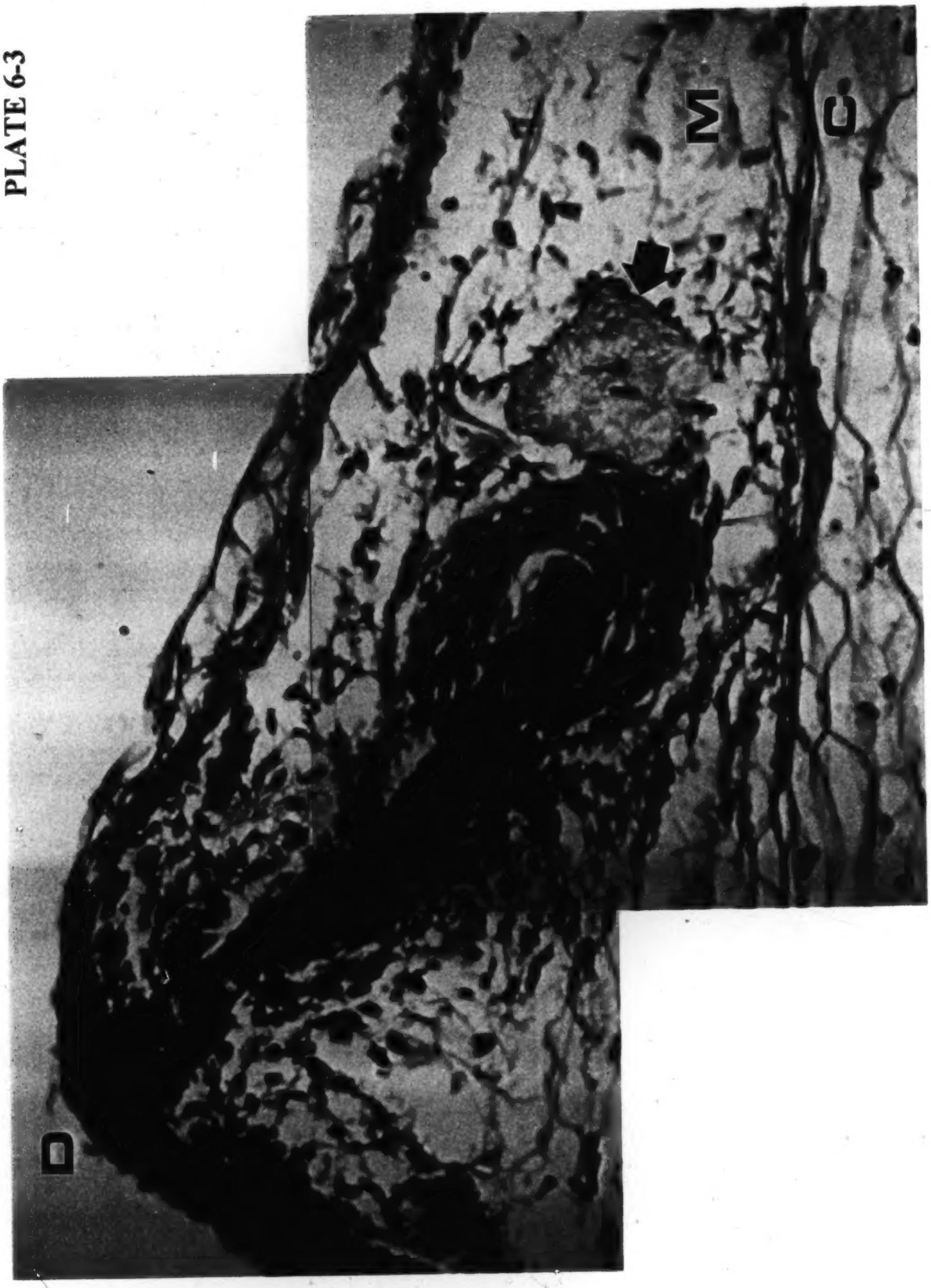


Plate 6-4A. Late Stage 54 oral epithelium and dorsal mesenchyme graft. A tooth germ with defined dentine core (curved arrow) surrounding the dental papilla (thin arrow). Dorsal mesenchyme (M). Dorsal surface is toward the top of the page. Stained with haematoxylin and eosin. Magnification (X 128).

Plate 6-4B. Late Stage 54 oral mesenchyme and dorsal epithelium graft. A well-defined tooth and enamel space (E). Dorsal mesenchyme (M). Suprarostral cartilage (C). Dorsal surface is toward the top of the page. Stained with haematoxylin and eosin. Magnification (X 128).

PLATE 6-4

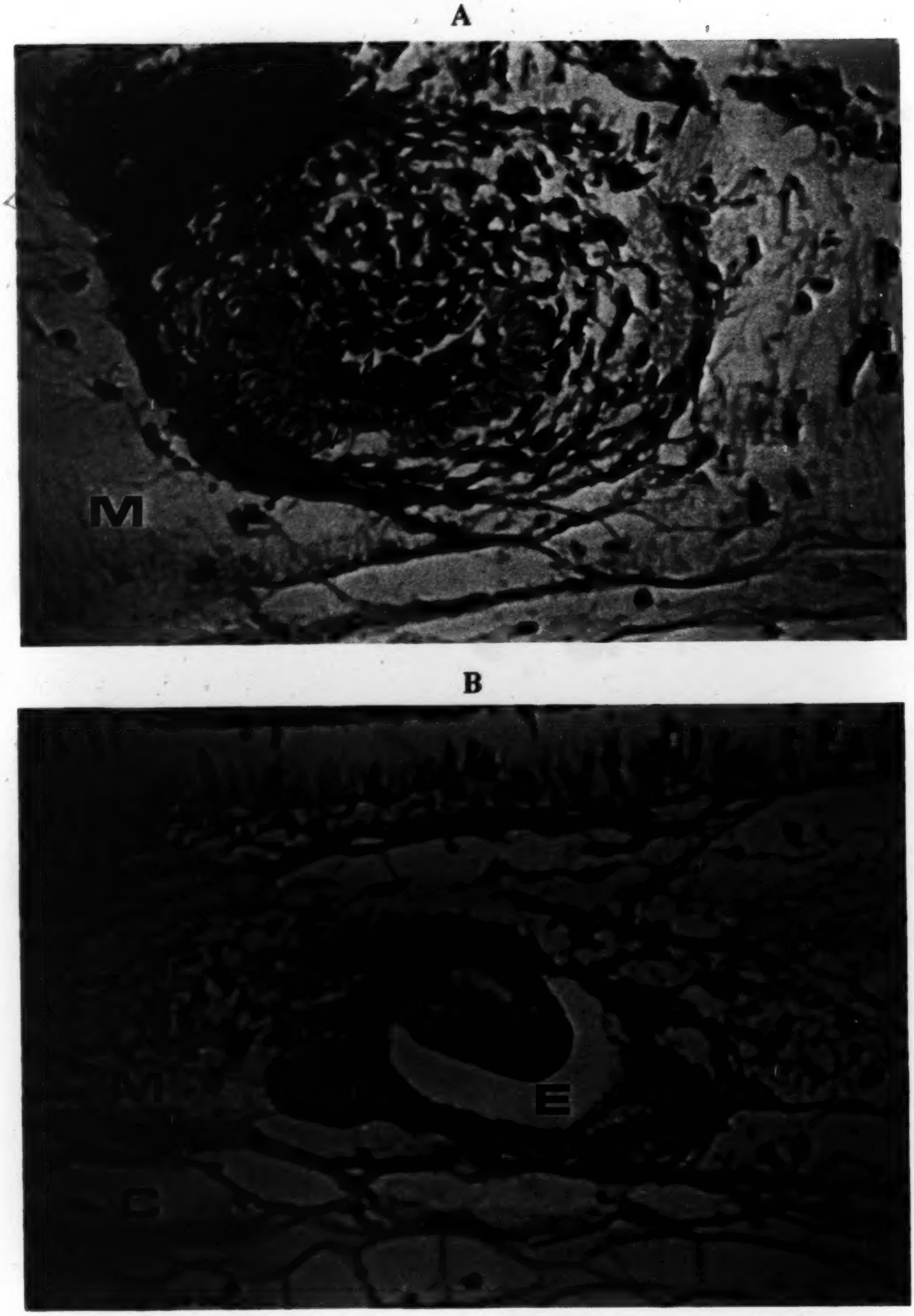


PLATE 6- 5

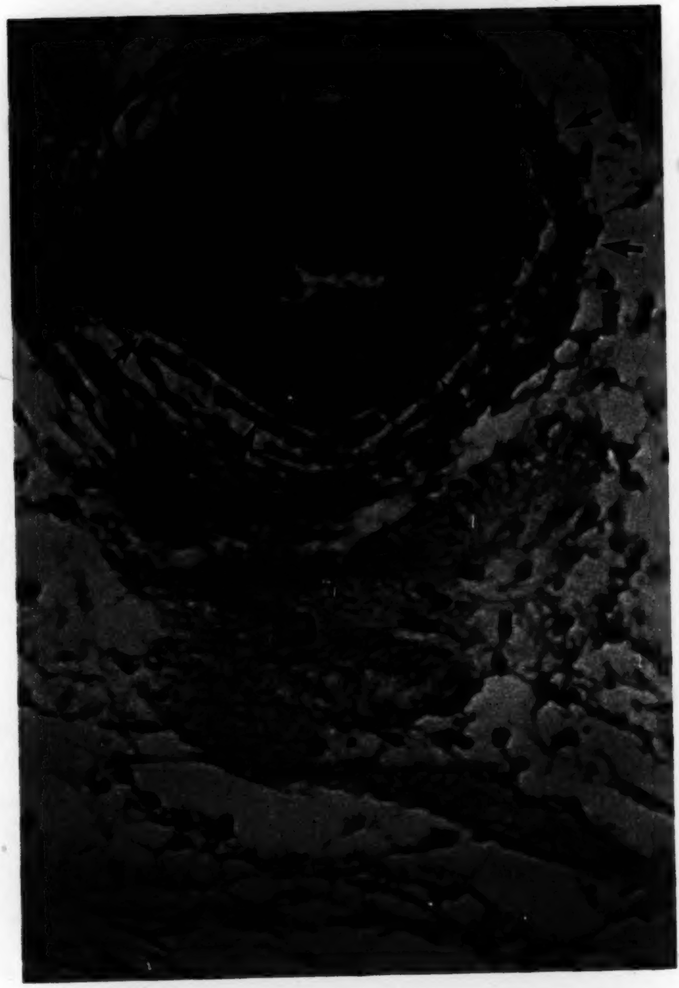


Plate 6-5. Late Stage 54 mandibular oral epithelium and maxillary oral mesenchyme graft. Irregular dentinoid mass (D) associated with epithelial portion of the graft (arrows). Dorsal surface is toward the top of the page. Stained with haematoxylin and eosin. Magnification (X 128).

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CHAPTER 7
CONCLUSION

This study describes the first longitudinal investigation of the developing embryonic dentition. Together with experimental work, it challenges the previous models on the development of the dentition--the Zahnreihen, Clone and New Progress Zone theories. In light of this descriptive and experimental evidence from larval *Xenopus laevis*, this thesis proposes the Odontogenic Field Theory for the development of the dentition. This model is consistent with current trends in evolutionary and developmental biology.

A. THE DEVELOPMENT OF THE EMBRYONIC DENTITION IN LARVAL *X. LAEVIS*

The development of the early dentition in *X. laevis* larvae features both form and freedom. That is, development proceeds within defined structural and genomic constraints of the odontogenic field and the initiation zones, yet it is the interaction at an epigenetic level of these that accounts for the pattern variability manifested in this dentition.

The *in vivo* record of embryonic dental initiation expands previous reports on the early development of the dentition in this animal (Nieuwkoop and Faber, 1967; Deuchar, 1975). It also challenges three important observations stated in the most extensive study on the development of this tadpole's dentition (Shaw, 1979, 1982, 1986). First, tooth germ initiation does not begin at the back of the jaw and then proceed toward the midline as claimed by Shaw. Rather, 4 to 8 primary tooth germs appear abruptly in the mesial two thirds of the jaw quadrant followed by the front to back addition of primaries in the distal third of the jaw. Second, the first tooth germs in the dental row do not develop in sequence. Their development pattern is irregular in the initial dental row (IDR) and generally sequential from front to back in the extending distal dental row (DDR). Lastly, the dentition does not develop *ab initio* as an alternating series of older even-numbered loci and younger odd-numbered loci. Clearly, alternation is a common feature of the larval dentition, but it is secondary to the developmental dynamics of the mesial dental row (MDR) and the distal dental row (DDR). Considering the variability noted in the dental initiation patterns, the discrepancies between the results of this study and that of Shaw underline differences between the longitudinal *in vivo* approach and static serial reconstructions.

B. MODERN THEORIES ON DEVELOPMENT OF THE DENTITION RECONSIDERED

The descriptive and experimental evidence on the developing embryonic dentition in larval *X. laevis* presented here is not consistent with the predictions of the modern theories on the development of the dentition.

Edmund's (1960, 1962, 1969) Zahnreihen Theory suggests that tooth initiation is the result of a morphogen travelling from the front of the jaw to the back, inducing successive dental primordia at regular intervals in space and time. The descriptive record of tooth germ initiation in the 44 jaw quadrants did not feature a single dental developmental pattern that was close to the pattern predicted by this theory. This descriptive evidence was supported experimentally. Excision of the middle third of the jaw three weeks before (Stage 45; 6 DPF) the histological evidence of the commencement of odontogenesis had no effect on the dentition that developed in the remaining mesial and distal jaw thirds.

That is, the path was removed along which the purported morphogens travel from a midline morphogen field generator to the back of the jaw. Instead, normal development characteristic of the DDR appeared in the distal segment clearly establishing the independence of this jaw segment from any developmental influence anterior to it. Similarly, the extirpation of presumptive dental row tissue from the distal third of the jaw 4 to 5 days prior to odontogenesis (Stage 53) and transplantation of this tissue outside the mouth to the dorsal surface resulted in dental development in every recoverable graft. In other words, dental initiation in the graft was not dependent on a purported oral morphogen released from a generator near the midline of the jaw.

Osborn's (1971, 1973, 1978, 1984, 1993) Clone Theory asserts that (a) discrete group(s) of ectomesenchymal cells give(s) rise to the entire dentition. The clone of cells grows in concert with the expanding jaw and dental initiation is controlled intrinsically, occurring once the necessary conditions of initiation are met outside zones of inhibition around developing teeth. However, the descriptive evidence of the embryonic dentition in *X. laevis* larvae was not consistent with that predicted by the Clone Theory. First, and most importantly, the length of the dental row when it first appeared far exceeded the jaw growth that occurred at that time, suggesting that the development of the dentition was not *directly* related to jaw expansion. More specifically, during the initiation of the IDR, the average amount of quadrant jaw growth was only 71 µm while the length of the IDR varied between 800-1500 µm. Moreover, Osborn's theory claims that interstitial growth between the first tooth germs in the dental row opens areas for interdental development. However, interdental growth between the primaries in the IDR at the time their secondaries appeared was negligible (5.5%). Second, the order of dental initiation was not consistent with that expected by the Clone Theory. Instead of primary tooth germs appearing sequentially out from the first tooth germ to arise in the mouth (i.e., the clone determinant), the initiation of primaries was variable and the first tooth germ did not appear at a consistent site. For that matter, multiple tooth germs often developed at the same time across the jaw, challenging the notion of a clone determinant. Experimental evidence supports these descriptive results. Extirpation of the middle third of the jaw early in development (Stage 45, 6 DPF) was performed in order to remove the growing clone or at least to interrupt its path. Osborn's theory predicts that this procedure should inhibit dental development in the distal third of the jaw. However, the surgery had no effect on the remaining mesial and distal jaw thirds, and the dental initiation proceeded normally. In the second experiment, distal third jaw tissue was transplanted to the dorsal surface at Stage 53. This tissue was excised 4 to 5 days prior to histological evidence of dental development in the anterior two thirds of the jaw. In other words, the grafted distal tissue should not have contained any dental clone tissue in it. However, dental development occurred in all recoverable grafts. These two experiments affirm the independence of dentition development from a specific site (i.e., a clone determinant) in the jaw.

Westergaard and Ferguson's (1986, 1987, 1990) New Progress Zone theory of dentition development emphasizes the primacy of the oral epithelium in dental patterning. This theoretical model features two components. First, an "early initiation stimulus" along with an inhibition mechanism "spread[s] in the epithelium." Second, this theory also envisions a proliferative epithelial "progress zone" that expands in concert with jaw growth, and that with either a cell lineage phenomenon and/or a positional information phenomenon epithelial cell division ceases, giving rise to new dental anlagen. In other words, this model of dentition development features elements of both the Zahnreihen and Clone theories. That is, on the one hand it has an initiatory signal travelling through the epithelium, yet on the other hand it functions like an epithelial clone of growing cells. However, the criticisms previously offered regarding these dental models also apply to the New Progress Zone model. Two observations from descriptive evidence on early dentition development in *X. laevis* larvae are relevant. First, the dental initiation patterns in the present study were not consistent with an initiatory stimulus spreading out from the first odontogenic site. For example, the initiation pattern of the primary tooth germs in the IDR was not sequential. Second, the large discrepancy between actual jaw growth and dental row development clearly argues that dentition development was not a simple and direct response to jaw growth. Moreover, the expected interstitial growth between primaries for the accommodation of interdental teeth was minimal. Experimentally, two results argue for the independence of dental initiation from a travelling stimulus in the epithelium or an expanding progress zone of epithelial cells. First, the dentition developed normally in regions lateral to the extirpated middle third of the jaw. Second, dental development occurred in grafts of presumptive dental row tissue that were moved to a site outside the mouth.

It must be emphasized that the modern theories on development of the dentition were constructed on reptilian models, and that the developing dentition in *X. laevis* larvae may prove to be *sui generis* in amphibians. Moreover, the level of investigation of this thesis was histological, and it is possible that a "covert" differentiation of the dentition at a molecular level might have occurred before any "overt" or histological differentiation. However, the modern models were constructed from standard histological data. The descriptive data in this study was observed at that level and failed to support these theories.

C. THE ODONTOGENIC FIELD THEORY

The Odontogenic Field Theory proposes that the dentition commences its development with the establishment of an epithelial odontogenic field (OF) in the jaw quadrant and that individual dental initiation zones (IZs) are competitively set up within it. Once a critical mass of OF epithelial cells is established, an IZ is created and a tooth germ emerges in the middle of it. Thus, dental initiation is locally controlled. The IZs also act as inhibitory zones not permitting the initiation of other tooth germs while active. This inhibitory effect later recedes, freeing up OF epithelium for the establishment of new IZs between the first tooth germs. As a result, IZs are instrumental in determining the spacing between tooth germs and ultimately the pattern of the dentition. The Odontogenic Field Theory is supported by descriptive and experimental evidence from the developing embryonic dentition of *X. laevis* larvae.

The spacing between the first tooth germs to appear in the OF suggests the existence of IZs prior to dental morphogenesis. More specifically, tooth germ clusters were not found in: (1) normal development in the IDR or the DDR, (2) the surgical triplets, or (3) the initial regeneration dental row (IRDR). That is, a mechanism spacing tooth germs apart was operative before their morphogenesis. Two lines of evidence support this contention. First, the null configuration rarely appeared within: (1) the normally developing dental row (3.2%), (2) the surgical triplets (5.4%), or (3) the IRDR (3.9%). Second, the average width of the IZ was nearly identical in normal development and in these two experimental contexts. In the former, it was suggested that in the IDR the solitary dental configuration reflected the marginal butting of IZs and that the interdental distance between tooth germs at initiation approximated the width of the IZ (218 µm). Similarly, in surgical triplets, the average interdental distance between the first tooth germs that appeared was 222 µm. And in experiments extirpating the presumptive complete dental row, the average interdental distance between the tooth germs expected to develop the solitary configuration in the IRDR was 213 µm.

The variability in the dental initiation patterns of the first tooth germs, points to the autonomous and competitive nature of the odontogenic initiation mechanism in the OF. In the normal development of the dentition, no two dental initiation patterns were identical in the 44 jaw quadrants examined. The number and position of primary tooth germs initiated, the order and time of their initiation and the interdental distance between them varied in each of the jaws, resulting in different dental configuration combinations. Similarly, the initiation patterns in surgical triplets was also variable. At least seven pattern types were identified, and pattern variability within each was also present. Finally, variable dental initiation patterns were also found with the primaries in the IRDR. No two IRDRs were identical with regard to primary tooth germ initiation number and position, initiation order and time, or initiation interdental distance.

The predictability of dental patterning based on the interdental distance of the primary tooth germs, and the lack of any significant change in dental patterning by experimental manipulation in neighboring regions of the jaw, both suggest that dental initiation is locally controlled and is not influenced by distant regions of the developing jaw and dental row. In normal development, the number of interdental tooth germs and the developmental time of the secondaries were related to the interdental distance of the bordering primaries. That is, the amount of available OF tissue at a site dictated dental initiation. These relationships were also seen in the surgical triplets. The number of tooth germs that developed in them correlated with their initial length. Similarly, the developmental time of the tertiary tooth germs in surgical triplets was related to the interdental distance of their bordering tooth germs. In other words, when a tooth germ was extirpated, the resultant initiation pattern reflected the surgically reconstituted developmental context of the immediate region.

Finally, regular dental patterning was also noted in the mesial and distal jaw segments after the middle third of the jaw quadrant was excised early in development (Stage 45; 6 DPF).

The early oral epithelium appears to initiate and organize the dentition. Two lines of evidence support this contention. First, dental initiation seems to be dependent upon the conditions of the OF epithelium, in particular the volume of available epithelium at the initiation site. During the course of the recording period the average primary tooth germ interdental distance in the DDR decreased at a regular rate. Through this same period, the average width of the oral epithelium in the region of the future dental row increased in a uniform manner. These observations are consistent with the notion that once a critical mass of OF epithelium was established dental initiation began. That is, with a thickening OF epithelium the critical epithelial volume for initiation was manifested within a narrower mesial-distal length of OF epithelium. Second, recombinations of presumptive dental tissue with non-dental tissue argues that the first site of dental patterning is in the oral epithelium. Early oral epithelium (early Stage 54) can instructively induce non-dental mesenchyme into odontogenesis while this ability is not present in dental mesenchyme at this same stage. The latter participates in odontogenesis only after it has been instructively induced by the oral epithelium (later Stage 54). In sum, initiation according to the Odontogenic Field Theory is a function of an epithelial critical mass.

This model bears important resemblances to previous theories dealing with the development of the dentition by Gillette (1955), Osborn (1971, 1978, 1993), Westergaard (1988a, 1988b), and Westergaard and Ferguson (1986, 1987, 1990). Dental initiation is intrinsically controlled at the site of initiation and independent of extrinsic factors from distant regions of the developing jaw and dental row. Moreover, an inhibitory or restrictive zone is associated with odontogenesis that is reminiscent of the long theorized "dental inhibitory zone." Finally, the dental initiation pattern reflects the interaction between the conditions of initiation and inhibition. However, three features distinguish the Odontogenic Field Theory from the previous models. First, the development of the dentition is not *directly* related to jaw growth. Second, the inhibitory/restrictive zone is established prior to actual dental morphogenesis. More specifically, an initiation zone is defined in the OF epithelium with a tooth germ later developing at its center. Third, the inhibitory effect of the IZ is transitory. That is, the IZ recedes soon after the beginning of dental morphogenesis, making OF epithelium available for other dental initiations in the interdental sites.

Oster and Murray (1989) noted that most models for embryonic pattern formation are built on the notions of local activation and lateral inhibition. Three features common to these models are seen in the odontogenic field theory. First, most biological patterns originate locally in an "organizing tissue" or "developmental field." Next, the activation of a structure is autocatalytic, being locally controlled, competitive, and dependent on a "sufficient tissue volume" or "critical domain size." Finally, lateral inhibition creates a "zone of influence" around an embryological structure and this zone precludes the establishment of other foci within it. Correspondingly, the Odontogenic Field Theory suggests that teeth are initiated in an odontogenic field through a competition between initiation zones which are regulated locally and develop when a critical volume of OF epithelium is established. The initiation zones inhibit the initiation of other tooth germs, and as a consequence provide a spacing mechanism between the first tooth germs across the early dental row.

At this point it is not possible to determine which molecular mechanisms are operative in the development of the tadpole dentition from the results of this thesis. However, the concept of lateral inhibition has recently seen biomolecular support through a process mediated by transmembrane proteins, *Delta* as a ligand and *Notch* as a receptor. In Drosophila, these proteins function in various cell fate determination events during oogenenesis, embryogenesis and metamorphosis (Muskavitch, 1994). More specifically, they are instrumental in a number of developmental contexts for the partitioning of cell fates in groups of equivalent cells (i.e., equivalency groups). In particular, during neurogenesis, *Delta*-expressing cells through a lateral signalling pathway inhibit neighboring cells from becoming committed to a neural fate (Heitzler and Simpson, 1993). Proneural gene products in the neuroblasts activate *Delta* transcription by binding to its promoter, thus establishing a lateral inhibition that ensures that neighboring cells become epidermoblasts (Kunisch *et al.*, 1994). However, Fortini and Tsakonas-Artavanis (1993) note that research in this area, particularly with *Notch*, has placed disproportionate attention on neurogenesis. Muskavitch (1994) also underlines the pleiotropic function of *Notch* and suggests a variety of models for the *Delta-Notch* mediation of lateral inhibition.

Notch and *Delta* have been identified in vertebrates. In *X. laevis*, an *X-Delta-1* homologue has been identified and the ectopic activity of this gene inhibits the production of primary neurons in the early embryonic nervous system (Chitnis *et al*, 1995). A similar manifestation occurs in the chick with *C-Delta-1* (Henrique *et al.*, 1995). Together these results suggest the conservation of the *Delta/Notch* lateral inhibition mechanism for the mediation of neurogenesis in vertebrates. More specifically, Myat *et al.* (1996) argue that *Notch* with ligands *C-Delta-1* and a *Delta*-related protein, *C-Serrate-1*, act in vertebrate neurogenesis through lateral inhibition. They speculate that *Notch* maintains a proportion of the neuroepithelial cells in an uncommitted stem-cell-like state.

Biomolecular investigations have implicated a number of transcription factors (*Msx-1*, *Msx-2*, *Egr-1*, *Lef-1*) growth factors (*BMP-2*, *BMP-4*, *TGF-β1*, *FGF-3*, *FGF-4*) and structural proteins (tenascin and syndecan) in dental development (Thesleff *et al.*, 1995a, 1995b). Previous research has established that a complex sequence of reciprocal epithelial-mesenchymal interactions, featuring both permissive and instructive interactions, is first defined in E9-E11 murine oral epithelium (Mina and Kollar, 1987; Kollar and Mina 1991). Though the exact identification of the molecular pathways directing dental fate determination and initiation remain unknown, two models are being considered.

In the first, *Notch 1*, *2* and *3* are expressed in the early oral epithelium, but by E11 all three are downregulated in the epithelial cells juxtaposed to the organizing dental mesenchyme (Mitsiadis *et al.*, 1995). It is noteworthy that at this time the mesenchyme instructively induces the epithelium (Mina and Kollar, 1987). Mitsiadis *et al.* acknowledge that an apparent role for *Notch* is to maintain the competence of undifferentiated cells (Coffman *et al.*, 1993; Fortini and Tsakonas-Artavanis, 1993), and they speculate that its expression in the oral epithelium blocks these cells from adopting an ameloblast fate.

A second developmental model implicates *BMP-4* and *Shh*. They are expressed in the early oral epithelium and provide the first defined molecular signs of dental initiation (Panaretto, 1993; Bitgood and MacMahon, 1995; Iseki *et al.*, 1996). *In vitro* studies reveal that *BMP-4* is involved in the epithelial signal by inducing the dental mesenchyme (Vainio *et al.*, 1993; Kratochwil *et al.*, 1996). *BPM-4* soaked beads can produce both morphological changes in mesenchyme consistent with early dental papilla organization and the expression of transcription factors in the dental signalling cascade (*Egr-1*, *Msx-1*, *Msx-2*, *Lef-1*). However, the exact relationship between *Shh* and *BMP* genes is not clear though Bitgood *et al.* (1995) suggest the latter is a target of the former.

The biomolecular investigation of dental development has been almost exclusively limited to murine studies and its molar series of three teeth. Unfortunately, this dental row is short and its teeth are initiated sequentially, making it difficult to assess the dynamics of initiation. On the other hand, the tadpole model offers an extended region in the mouth (IDR) where 4-8 tooth germs are initiated abruptly in what appears to be a developmental field (OF). As a result, this region in the medial two-thirds of the jaw quadrant system provides an accessible site to investigate the partitioning of cell fates in an equivalency group, the odontogenic field. The molecular labelling of *Notch, Delta, Shh* and *BMP* may provide patterns reflecting the dental initiation zones early in the development of this dentition.

D. EVOLUTIONARY IMPLICATIONS

Hall (1983, 1990, 1992) notes there is a late 20th century renaissance in the study of developmental biology and evolution as seen with the recent emergence of the subdiscipline of developmental evolutionary biology. In particular, Gilbert *et al.* (1996) calls attention to the rediscovery of the morphogenetic field and its part in the "new synthesis" of evolutionary and developmental biology. Once marginalized, this embryological concept is now being viewed as a major unit of ontogeny whose modifications could account for significant evolutionary change. This approach is also emerging in dental studies (Slavkin and Diekwisch, 1996) and consideration is being given to the possibility of the "odontogenic homeobox code" (Vastardis *et al.*, 1996).

This descriptive and experimental study on larval *X. laevis* proposes that the dentition develops within a morphogenetic field; specifically, the odontogenic field. From an evolutionary perspective, the number of features noted in the development of this morphogenic field could be considered homologous. However, the variability noted in this animal's embryonic dental patterning suggests dental homology in similar lower tetrapods does not reside at the level of the tooth itself. Rather, it resides at the level of the basic structures and processes of odontogenesis: the OF epithelium, the necessary conditions for the initiation of an IZ, and the inhibitory/restrictive conditions associated with the IZ. The evolutionary conservation of these homologous processes can be seen in the primacy of the dental epithelium in dental patterning as noted in both the tadpole and the mouse (Mina and Kollar, 1987; Kollar and Mina, 1991; Chapter 6). However, the limits of this study must be underlined. The molecular mechanisms that establish the OF and IZs are not known, and biomolecular investigation is necessary in order to confirm fully the odontogenic field as a morphogenetic field.

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